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The purpose of our research has been to delineate mechanisms of neuronal communication in the mammalian brain We have studied rapid mechanisms of communication, including electrical transmission and chemical synapses, with an emphasis on local interactions among cortical and hypothalamic neurons. In the hippocampus, we are completing our studies concerning the effects of altered osmolality of the extracellular fluid on synchronous bursting of population spikes in low-[Ca^{2+}] solutions (chemical synapses blocked). Electrophysiological studies on hypothalamic neurons have primarily evaluated the role of excitatory amino acids in fast synaptic transmission in the supraoptic, paraventricular and suprachiasmatic nuclei. Intracellular recordings have allowed direct analyses of EPSPs, and single-electrode voltage-clamp experiments have permitted analyses of synaptic currents. These experiments have provided direct evidence that glutamate is the primary fast excitatory transmitter throughout the hypothalamus.

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P.I.--F.E. Dudek
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1. RESEARCH OBJECTIVES

The original application was aimed at understanding electrical mechanisms of neuronal communication with particular emphasis on their possible synchronizing actions. Although this work has relevance to epileptogenesis, our overall goal has been to understand the electrical mechanisms responsible for synchronizing neuronal activity in the hippocampus. In the final year of this grant we finished a series of experiments concerning the effects of osmolality on synchronous activity in the hippocampus under conditions where chemical synaptic transmission was blocked.

Throughout much of this grant period, after extensive discussions with Air Force administration, we have undertaken a series of electrophysiological experiments aimed at understanding fundamental mechanisms of synaptic transmission in the mammalian hypothalamus. The initial studies have been performed on the magnocellular neuroendocrine system (i.e., the paraventricular and supraoptic nuclei), but our recent work has also been aimed at the suprachiasmatic nucleus. We should emphasize that the paraventricular nucleus, in addition to containing oxytocin and vasopressin neuroendocrine cells, also contains neuroendocrine cells that regulate secretion of corticotropin-releasing hormone, which mediates the stress response. The suprachiasmatic nucleus regulates circadian rhythms. Our goal has been to understand the electrophysiology of these critical hypothalamic nuclei, and in particular, to delinate the role that excitatory amino acids play as neurotransmitters in these systems.

2. STATUS OF THE RESEARCH

A. Effects of osmolality on synchronous activity of hippocampal neurons: evidence for an important role of non-synaptic mechanisms.

In the previous progress report we outlined in some detail the experiments we had been ertaking with low-[Ca^{2+}] solutions that were aimed at examining non-synaptic mechanisms of ronal communication. Although we had performed many experiments on this project by the .e ad wrote the previous progress report, additional experiments were necessary in order for us to re a manuscript. A short paper on this work is enclosed along with an abstract, and a full-length paper is currently in preparation.

B. Evidence that excitatory amino acids (EAAs) are the primary fast synaptic transmitter in the hypothalamus.

For several years we have been studying the role of glutamate, or some other excitatory amino acid, as a neurotransmitter in the hypothalamus. Our initial experiments involved intracellular electrophysiological studies of the supraoptic nucleus, which have since been followed by extensive experimentation in the paraventricular nucleus. More recently we have undertaken a similar series of studies on the suprachiasmatic nucleus.

1. Supraoptic nucleus

A detailed description of our experimental findings was provided in the last progress report. Although a preliminary study has been published (Gribkoff and Dudek 1988), a longer manuscript has since been completed and is now published. A reprint of this paper is enclosed.

2. Paraventricular nucleus

Research in this area has followed two primary lines of investigation. The first was to define rigorously the electrophysiological properties of paraventricular neurons. Secondly, we analyzed the effects of excitatory amino acid antagonists on EPSPs from these different types of hypothalamic neurons. A summary of the electrophysiological properties of paraventricular neurons is provided in Hoffman et al. (1989), which was presented to the Society of Neuroscience. Two manuscripts are now in preparation concerning this work. A summary of the data on excitatory amino acid antagonists is available in Wuarin and Dudek (1989). The data in this abstract are also currently being prepared for publication.

3. Suprachiasmatic nucleus

An extensive analysis of the possible role of excitatory amino acids in synaptic transmission has been undertaken in the suprachiasmatic nucleus. Intracellular recordings have been obtained from many neurons in this area and the available data indicate that non-NMDA receptors (as in the paraventricular nucleus) mediate fast synaptic transmission both from retinal and non-retinal input. A manuscript on this work has been submitted for publication.

4. Conclusion

The research supported by this grant has provided important new information concerning the role of non-synaptic mechanisms in synchronization of electrical activity of the hippocampus. Other studies have evaluated the role of excitatory amino acids in synaptic transmission in the hypothalamus. Our data provides strong evidence that excitatory amino acids are the primary fast excitatory transmitter throughout the hypothalamus.

3. PUBLICATIONS

A. Refeered Publications

Dudek, F.E., Tasker, J.G. and Wuarin, J.P. (1989) Intrinsic and synaptic mechanisms of hypothalamic neurons studied with slice and explant preparations. J. Neurosci. Meth. 28:59-69 (reviewed symposium chapter).

Gribkoff, V.K. and Dudek, F.E. (1990) The effects of excitatory amino acid antagonists on synaptic responses of supraoptic neurons in slices of rat hypothalamus. J. Neurophysiol. 63:60-71.

B. Symposia and Book Chapters

Dudek, F.E., and Traub, R.D. (1989) Local synaptic and electrical interactions in hippocampus: experimental data and computer simulations. Neural Models of Plasticity: Theoretical and Empirical Approaches, Ed. by W.O. Berry and J. Byrne, Academic Press, 378-402.

Dudek, F.E., Wuarin, J.P. and Kim, Y.I. (1990) Evidence that excitatory amino acids mediate fast synaptic transmission in the hypothalamus. Invited review article for a supplemental issue of Biomedical Research in honor of Prof. Y. Sano (in press).

C. Abstracts

Tasker, J.G. and Dudek, F.E. (1989) The effects of osmolality on synchronous bursting in the absence of chemical synaptic transmission in hippocampal slices. Soc. Neurosci. Abstr. 15:701, #284.7.

Kim, Y.I. and Dudek, F.E. (1989) Antagonism of fast excitatory postsynaptic potentials in suprachiasmatic nucleus neurons by excitatory amino acid antagonists. Soc. Neurosci. Abstr. 15:1088, #431.15.

Hoffman, N.W. Tasker, J.G. and Dudek, F.E. (1989) Comparative electrophysiology of magnocellular and parvocellular neurons of the hypothalamic paraventricular nucleus. Soc. Neurosci. Abstr. 15:1088, #431.16.

Wuarin, J.P. and Dudek, F.E. (1989) Contrasting effects of NMDA and non-NMDA antagonists on fast EPSPs in neurons of the paraventricular nucleus. Soc. Neurosci. Abstr. 15:1088, #431.17.

4. PROFESSIONAL PERSONNEL

Dr. Neil Hoffman
Dr. Yang I. Kim
Dr. Andre Gbenaus
Dr. Jeffrey Tasker
Dr. Jean-Pierre Wuarin

5. INTERACTIONS

Drs. Dudek, Gillette, van den Pol and Rea participated in a workshop at the Winter Conference on Brain Research during January, 1990. The purpose of this workshop was to present our preliminary work to the Neuroscience community in a workshop format and to exchange ideas among our group. The scientific goal of the workshop was to provide a cellular analyses of the suprachiasmatic nucleus.

6. NEW DISCOVERIES, INVENTIONS OR PATENT DISCLOSURES--none

7. OTHER STATEMENTS

These projects will be continued under the auspices of a new grant on the suprachiasmatic nucleus. We intend to continue some of the electrophysiological studies of hippocampal neurons, and to focus our efforts on the suprachiasmatic nucleus. We will, however, complete our experiments on the paraventricular nucleus, and we have initiated studies in the medial preoptic area.

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Local Synaptic and Electrical Interactions in Hippocampus: Experimental Data and Computer Simulations

F. Edward Dudek and Roger D. Traub

I. Introduction

An understanding of neuronal plasticity requires three types of information. First is a picture of how brain function proceeds at a given time and in the absence of external disturbances that modify the underlying physical substrate of the brain itself. Such a picture must integrate results on membrane biophysics and single-neuron electrophysiology with data on synaptic circuitry and other relevant interactions between neurons. Thus a single model must include experimental results ranging from the properties of membrane channels to the behavior of neuronal circuits. Second, we must have a picture of how experiences (i.e., inputs to the brain) are transformed into changes in specific physical parameters within the brain. How does experience excite particular cellular activities, and then how do these lead to long-lasting modifications in channels, synapses, and neuronal populations? Finally, given that certain parameters of the brain have indeed been altered, by whatever mechanisms, how is brain function now different than it was before? Thus, in what meaningful ways does the system as a whole alter its activities once particular neurons and their synapses have in fact been modified? In this chapter, we shall deal mainly with an example of the first type of problem. We present experiments and computer simulations aimed at elucidating the collective behavior of populations of hippocampal neurons, in the absence of so-called plastic changes.

Two dominant features of the evolutionary progression from invertebrates to lower mammals to humans have been the increase in the number of neurons in the brain and the enhancement of both the number and complexity of local neuronal interactions. Numerous model systems for the study of synaptic plasticity and learning in lower animals, such

Liberman, A. M., Cooper, F. S., Shankweiler, D. P., and Studdert-Kennedy, M. (1967). *Psychol. Rev.* 74, 431.
Llinas, R., and Yarom, Y. (1981). *J. Physiol. (London)* 315, 569.
Suga, N. (1984). *Trends NeuroSci.* 7, 20 (1984).
Sullivan, W. E., and Konishi, M. (1986). *Proc. Natl. Acad. Sci. U.S.A.* 83, 8400–8404.
Tank, D. W., and Hopfield, J. J. (1986). *IEEE Trans. Circuits and Systems* 33, 533–541.
Tank, D. W., and Hopfield, J. J. (1987a). *Proc. Natl. Acad. Sci. U.S.A.* 84, 1896–1900.
Tank, D. W., and Hopfield, J. J. (1987b). *Proc. Int. Conf. Neurol Networks, 1st* IV,, 455–468.

as gastropod mollusks (e.g., *Aplysia*, *Limax*, and *Hermissenda*), have provided important information about how individual neurons and their biophysical properties operate within neural circuits to generate simple behaviors and behavioral modifications. Although these studies (many reviewed in this volume) have produced valuable information that will almost certainly apply to the mammalian brain, a distinct feature of the human brain that is not represented in simple "model" systems is the enormous integrative complexity via local neuronal interactions. These integrative local mechanisms include (1) recurrent inhibition and excitation mediated by chemical synapses, (2) electrotonic coupling via gap junctions, (3) electrical field effects (ephaptic transmission), which arise from current flow through the extracellular space, and finally (4) changes in the concentration of extracellular ions, such as K^+ and Ca^{2+}. Each of these mechanisms has been found to occur in "simple" systems, and information from these preparations has been extremely valuable in understanding biophysical principles. However, when operating *together* and involving large numbers of cells simultaneously, these neuronal interactions confer an enormous degree of complexity on a neuronal network. This is true even with relatively simple forms of experimental analysis such as input–output relations and the effects of previous activity. During the normal functions of the brain, it is likely that the tremendous number of neurons in each structure combined with their large array of integrative mechanisms leads to new physiological processes not found in "simple" systems. Even a rudimentary understanding of this complexity requires computer models of neural networks using neurons with realistic (if not precise) electrophysiological properties. The approach of electrophysiological experimentation involving membrane conductances, whole nerve cells, and neuronal populations *combined* with computer models of the appropriate magnitude and detail has the potential to reveal the collective or *emergent* properties that occur in large ensembles of neurons characteristic of the mammalian brain. Some of the collective properties may not be obvious (and may even be counterintuitive) from considerations simply of the properties of simple cells and of interactions between pairs of cells.

Many forms of neuronal plasticity, such as long-term potentiation (LTP), should lead to alterations in local neuronal interactions. The relative importance of each form of local interaction depends on the particular situation. Chemical synapses, whether from long-distance projections or local neuronal circuits, are considered the basis for neural integration under normal conditions. On the other hand, certain abnormal conditions, such as epileptiform activity, provide insights into a broader range of neuronal interactions—a range that includes, but is not limited to, chemical synapses. Epileptiform bursting is characterized by extreme synchrony and hyperactivity, and it represents a useful model for studying how such synchrony can arise in the first place and how

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actions. Although local chemical synapses are known to play a critical role in the spread and synchronization of epileptiform activity, electrical interactions are also likely to be important.

As background for this chapter, we will first briefly review electrophysiological data and computer simulations concerning the characteristics of single pyramidal cells and the network properties associated with a sparse system of local excitatory synapses. Our primary aim, however, will be to describe recent efforts to incorporate electrical interactions, particularly field effects (i.e., ephaptic transmission), into this network model so that it reproduces and predicts electrical behavior of complex neuronal populations from known properties of single cells and synaptic interactions between neuron pairs. Our starting point in the results section will be the rather surprising observation obtained by several groups that exposure of hippocampal slices to low-[Ca^{2+}] solutions, which completely block spike-dependent chemical synaptic transmission and also increase membrane excitability, leads to synchronous bursting. After briefly reviewing data that suggest an important role for electrical field effects in this synchronization process, we will describe simulations that reproduce this behavior and provide a quantitative, conceptual framework for understanding how this occurs. Next, we will review further data and modeling efforts to incorporate simultaneously both local excitatory synapses and electrical interactions into models of synchronous bursting by hippocampal neurons. The conclusions that derive from this combined approach, in turn, suggest new physiological processes that now appear difficult to analyze experimentally, but which may be studied further with an appropriate computer model. Conversely, computer models provide important insights into those experimental issues that are the most critical for further research.

A. The Hippocampus

The hippocampus has long been considered important for learning and memory (see Chapters 12 and 13, this volume), and it is also particularly sensitive to epileptic seizures. One important experimental advantage of this structure is that most of the projection neurons are of a single type, pyramidal cells. Although considerable information was and is available from research in intact animals, the use of slice preparations in the last decade or two has greatly expanded our knowledge of membrane conductances, whole-cell electrophysiology, local interactions, and projections to and from the hippocampus. These data have recently been supplemented by results from isolated dissociated neurons. This large body of information, at many levels and from numerous laboratories, has been instrumental in the development of a model of hippocampal network properties.

B. Electrophysiology of Single Pyramidal Cells

A wide range of intracellular studies in the late 1970s and throughout the 1980s have yielded a general picture of the ionic conductances of CA1 and CA3 pyramidal cells. Biophysical analyses of pyramidal cells have indicated that their electrotonic length corresponds to about one space constant (Johnston and Brown, 1982; Brown and Johnston, 1982). Intracellular recordings have shown that hippocampal pyramidal cells possess dendritic Ca^{2+} conductances (Wong *et al.*, 1979), that their Ca^{2+}-mediated depolarizing afterpotentials lead to intrinsic bursts (Wong and Prince, 1981), and that spike bursts are followed by a Ca^{2+}-activated K^+ conductance (Brown and Griffith, 1983). Although the kinetics of the voltage-dependent conductances and the precise location and density of each channel type are still unknown (however, see Kay and Wong, 1987; Numann *et al.*, 1987), enough information has been available to construct a single-cell computer model that reproduces many of the known properties of hippocampal cells. In the model, each neuron contains 19–28 compartments (depending on the presence or absence of apical dendritic branching), including one for the soma. Each compartment has a membrane capacitance and leakage conductance, and is connected to adjacent compartments through a resistance comparable to that of the intracellular medium. Dendrites are modeled as equivalent cylinders (Rall, 1962), with a single or branched apical dendrite and a single basilar dendrite. The apical and basilar dendrites are 1.0 and 0.8 space constants, respectively, in electrotonic length. The soma and occasionally one dendritic compartment have active conductances capable of burst generation (Traub, 1982). As a minimum, the model of a single neuron contains (1) a Na^+ current for action potential generation, (2) a Ca^{2+} current to produce spike depolarizing afterpotentials and slow action potentials, and to control a slow Ca^{2+}-dependent K^+ current, (3) a fast voltage-dependent K^+ current with voltage-dependent inactivation, which represents a hybrid of the delayed rectifier and A-currents, and (4) a Ca^{2+}-dependent K^+ current that produces a long hyperpolarization after a spike burst. In this form, the model reproduces faithfully a wide variety of spontaneous and current-evoked events characteristic of hippocampal pyramidal cells.

C. Local Neuronal Interactions

It has long been established that antidromic or synaptic activation of hippocampal pyramidal cells is followed by powerful gamma-aminobutyric acid (GABA) mediated recurrent inhibition, and that blockade of this system leads to synchronous bursting throughout the hippocampal population. Dual intracellular recordings have shown unequivocally that recurrent excitation is present among CA3 pyramidal cells, although the

connectivity seems to be relatively sparse (MacVicar and Dudek, 1980; Miles and Wong, 1986). Several studies have provided evidence that recurrent excitation is the primary contributor to synchronization of epileptiform bursts in the CA3 area (Johnston and Brown, 1981; Traub and Wong, 1982). Several independent lines of evidence have suggested the presence of electrotonic junctions, but these also appear to be sparse (see Dudek *et al.*, 1983, 1986, for review) and their role in synchronization remains uncertain. Differential recordings of transmembrane potential have shown that electrical field effects (i.e., ephaptic transmission) occur during synchronous firing or so-called "population spikes" (see Dudek *et al.*, 1986, for review). Thus, hippocampal neurons can communicate with their neighbors through several possible mechanisms, although this chapter will focus primarily on electrical field effects.

D. Recurrent Excitation and Synchronous Bursting

Considerable evidence from research in the 1970s had suggested that local excitatory synapses play a critical role in synchronization when inhibitory synapses are blocked with penicillin or picrotoxin (Ayala *et al.*, 1973). Biophysical studies supported the hypothesis that a large synaptic conductance is responsible for the depolarization shift that occurrs in CA3 pyramidal cells when inhibition is pharmacologically blocked (Johnston and Brown, 1981). Nonetheless, the initial studies with dual intracellular recording suggested that only a few percent of the cell pairs, at most, were connected by excitatory synapses (MacVicar and Dudek, 1980). Traub and Wong (1982) used computer simulations with a 100-neuron network, which contained realistic single-cell electrical properties and excitatory synaptic interconnections, and were able to reproduce bursting behavior at the single-neuron and hippocampal-population levels. Conceptually, the model involved a cascade effect whereby a small group of excited neurons activated the entire population in progressive stages of synaptically induced bursting. Connectivity was too sparse for any small group of cells to excite the entire population directly. A critical assumption was that recurrent excitatory synapses were powerful enough so that bursting in one cell would evoke bursting in a connected postsynaptic cell, provided the postsynaptic cell was not simultaneously inhibited or refractory from a recent burst. The model could account for several features of the electrophysiological data, such as the 50- to 100-msec latency from a local stimulus to a population burst. The model was also predictive; for example, it suggested that activation of a single CA3 pyramidal cell should—in some cases—initiate a synchronous population burst. Subsequent studies in picrotoxin-treated slices indeed showed that in about one of three cells, an evoked spike burst in a single cell could trigger a burst from the entire CA3 population (Miles and Wong, 1983). Furthermore, dual intracellular recordings in CA3 have directly shown

that excitatory synapses are indeed powerful enough for burst transmission to occur between monosynaptically connected cells (Miles and Wong, 1987a). These and other data provided overwhelming evidence in favor of a critical role for chemical synapses in synchronizing the activity of CA3 pyramidal cells. This model did not require any form of electrical or ionic interaction to reproduce—at least qualitatively—several well-established electrophysiological observations.

II. Results

A. Synchronization through Electrical Interactions Alone

1. *Electrophysiological Data*

a. Synchronous Bursting in Low-[Ca^{2+}] Solutions. Exposure of hippocampal slices to low-[Ca^{2+}] solutions, which demonstrably block chemical synaptic transmission, can lead to evoked and spontaneous bursts of population spikes (Taylor and Dudek, 1982b; Jefferys and Haas, 1982; Konnerth *et al.*, 1984). Although *spontaneous* release of synaptic "quanta" can still occur in this solution, spontaneous release that is not coupled to action potentials cannot be an effective synchronizing mechanism. These electrophysiological data therefore provide strong evidence that mechanisms other than chemical synapses can synchronize the firing of CA1 pyramidal cells. With further reductions in the concentration of divalent cations, the dentate granule cells and CA3 pyramidal cells can also fire synchronous action potentials (i.e., population spikes) with chemical synaptic transmission blocked (Snow and Dudek, 1984a). Although an important contribution by shifts in extracellular K^+ has been proposed, particularly for the spread of these synchronized bursts (Konnerth *et al.*, 1984, 1986; Yaari *et al.*, 1986), electrical interactions between neurons almost certainly play a critical role as a synchronizing mechanism to generate the large population spikes in low-[Ca^{2+}] solutions.

b. Electrotonic Coupling versus Electrical Field Effects. Either electrotonic junctions or electrical field effects could in principle synchronize hippocampal pyramidal cells when chemical synapses are blocked in low-[Ca^{2+}] solution. Two types of evidence suggest that electrotonic coupling via gap junctions cannot by itself account for the synchronization. First, all experimental measures of electrotonic coupling suggest that the cells do not form the interconnected syncytium that would be necessary for extensive synchronization to occur. The available data argue that many hippocampal neurons are not coupled, and that those that are coupled are only connected to a few other cells to generate small groups or clusters. Second, although events similar to what one would expect of elec-

trotonic coupling potentials (i.e., fast prepotentials, see Fig. 1B) can be observed in some pyramidal cells when chemical synapses are blocked, in most neurons these events are rare and it is difficult to reveal them during spontaneous discharges. Although particularly strong or particularly weak electrotonic junctions to an impaled neuron might not lead to coupling potentials after presynaptic spikes, these observations strongly argue that electrotonic coupling alone cannot account for the profound synchronization of action potentials routinely observed in low-$[Ca^{2+}]$ solutions.

Although electrical field effects have long been considered a possible mechanism for synchronizing cortical neurons (see Dudek *et al.*, 1986, for discussion), this hypothesis fell into considerable disfavor throughout the 1970's. However, studies in other neuronal systems, particularly the Mauthner cell in goldfish (e.g., see Korn and Faber, 1979), clearly showed that the electrical field from an action potential in one neuron can influence the excitability of other neurons if certain morphological considerations are applicable. Because of the particular anatomical arrangement in the Mauthner cell system of the goldfish, action potentials can produce inhibitory field effects. Differential recording (intracellular minus extracellular) of transmembrane potential during synchronous firing (i.e., population spikes) of hippocampal neurons has revealed field-effect depolarizations when chemical synapses were blocked (Fig. 1C and D). In fact, whenever the criteria for accurate measurement of transmembrane potential with differential recording techniques are met, field-effect depolarizations can be detected during population spikes and are associated with an increase in membrane excitability (Taylor and Dudek, 1984a,b). Thus, hippocampal population spikes can have an excitatory influence on inactive cells in the population, but it is unclear how small a group of hippocampal neurons must fire synchronously to cause a nearby inactive neuron close to threshold to fire an action potential. The fact that the previously reported field effects are inhibitory in the Mauthner cell system and excitatory in the hippocampus is readily explained by the different geometrical arrangements of the interacting neurons. The existence of field-effect depolarizations during differential recording and simultaneous negativities during single-ended intracellular recording, combined with the general lack of intracellularly recorded positive transients (i.e., electrotonic potentials), imply that field effects are more likely to synchronize action potentials in this preparation than is electrotonic coupling (Taylor and Dudek, 1984a,b; Taylor *et al.*, 1984; Yim *et al.*, 1986).

2. *Computer Modeling*

The observation of synchronous firing without chemical synapses suggested that electrical interactions, particularly field effects, need to be included in models of synchronous firing by hippocampal pyramidal cells. Although incorporation of electrotonic junctions into the computer

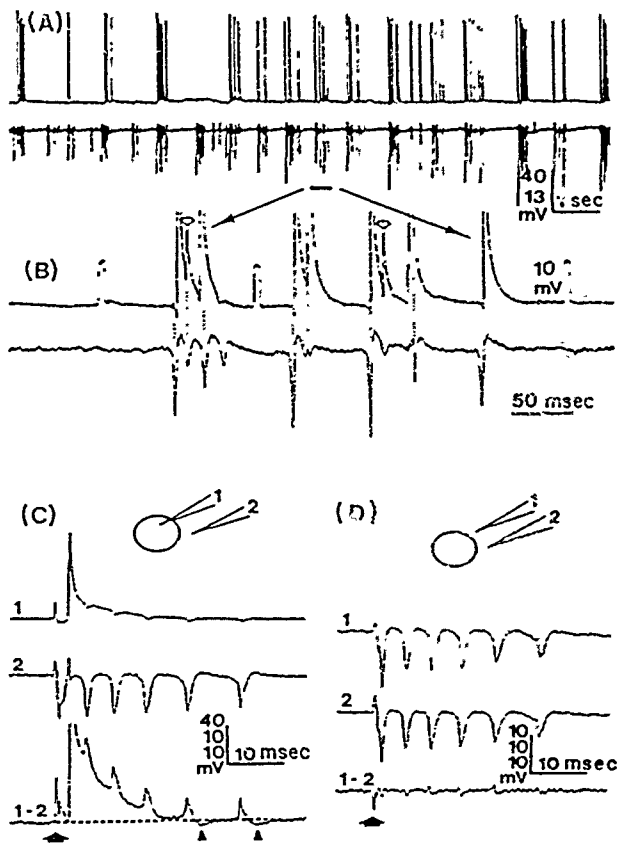

Figure 1.
Synchronous bursting of hippocampal pyramidal cells in low-$[Ca^{2+}]$ solutions that block chemical synapses. (A) Spontaneous bursts of synchronous action potentials recorded from CA1 pyramidal cells in a hippocampal slice bathed 4 hr in a solution containing 2.3 mM Mn^{2+} and 0.5 mM Ca^{2+}. The solution rapidly blocked chemical synaptic transmission to CA1 pyramidal cells. Intracellularly recorded action potentials (top trace) occurred synchronously with population spikes in the extracellular record (lower trace). (B) Part of the records from (A) are expanded, as indicated by the bar and arrows. Open arrows point to subthreshold depolarizations, which resemble fast prepotentials. (C) Differential recording of transmembrane potential. As indicated in the inset, the intracellular trace (1) minus the extracellular trace (2) represents a measure of transmembrane potential (1 − 2). Field-effect depolarizations, which were only apparent in the differential recording, occurred synchronously with population spikes in the extracellular record. The dashed line shows resting potential. (D) Extracellular control. The intracellular electrode was withdrawn about 5 μm to provide evidence that the extracellular electrode in (C) was close to the impaled cell. Differential recording under these conditions revealed no depolarization during the population spikes (bottom trace), thus indicating that the differential recording in (C) accurately measured transmembrane potential. [Reproduced from Taylor and Dudek (1982), with permission. Copyright 1982 by the AAAS.]

simulations was comparatively simple, since they can be represented electrically as simple low-resistance pathways between neurons, the addition of field effects was considerably more difficult. An analytical approach—for example, calculating extracellular potentials from Poisson's equation applied to an idealized set of current sources—was rejected because it did not allow appropriate interplay between transmembrane currents and extracellular currents. The algorithm must allow for the possibility that transmembrane currents flowing through the extracellular medium will alter transmembrane potentials, which in turn will affect voltage-dependent transmembrane currents (Fig. 2). A network of neurons with electrical field effects between individual elements will therefore possess "cooperative" properties, since extracellular current flow and the electrical behavior of the neurons continuously affect each other. An approach is required, therefore, in which neuronal behavior and extracellular current flow are coupled together on the time scale over which such properties actually change. We have produced such an approach (Traub *et al.*, 1985a,b) wherein the differential equations for the neurons and the linear equations for extracellular current flows are coupled every integration step (i.e., every 50 μsec).

a. Structure of the Model. The initial network for analyzing electrical field effects was an extrapolation of the 100-cell model used to study synaptic interactions. It is necessary that the model of each cell include dendrites, so that current loops can form across different regions of cell membrane. The extracellular space is simulated as a resistive three-dimensional lattice where two dimensions represent a layer of pyramidal cells, and the third dimension is for vertical layers corresponding to dendritic compartments. Although the initial model contained 100 neurons with a branched apical dendrite and 27 compartments at 19 levels, the most recent one contains 2000 cells with unbranched apical and basilar dendrites (Fig. 3). Each dendritic compartment is 0.1 space constant in electrotonic length. The different levels in the extracellular lattice are not evenly spaced, in order to match the physical coordinates of the lattice and the electrotonic coordinates of the model neurons (Rall, 1962). Because of dendritic branching in hippocampal neurons, the length of a segment of dendritic cylinder corresponds to a smaller length of actual space as one moves away from the soma (Rall, 1962). Currents flow inside the neurons and across membranes to corresponding points in the extracellular lattice and through the lattice. Two opposite faces of the lattice are grounded (i.e., are connected to points fixed at zero potential) to represent a slice with two faces in contact with a medium whose resistivity is small compared to tissue resistivity and whose other faces are closed to current flow. Extracellular resistivity is increased in the vicinity of the cell body layer in accord with the results of Jefferys (1984). In present implementations, there are two cells for each section through

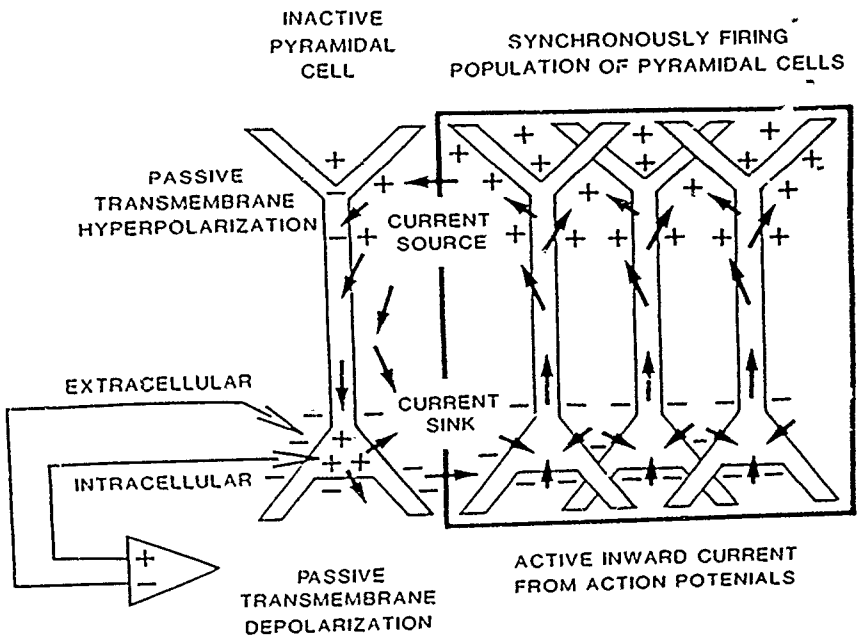

Figure 2.
Hypothetical mechanism of electrical field effects. The square (at right) represents a population of synchronously firing pyramidal cells. Inward current at the cell bodies from a population spike by these neurons causes a current sink or extracellular negativity at the soma of the inactive pyramidal cell (left). The sink in the cell body layer and the source in the dendrites are associated with current flow (indicated by arrows). Differential recordings from the intracellular and extracellular microelectrodes show field-effect depolarizations in the cell body of an inactive hippocampal neuron (i.e., one that is not firing when the population is active). [Reproduced from Dudek *et al.* (1986). Role of electrical interactions in synchronization of epileptiform bursts. *In* "Basic Mechanisms of the Epilepsies" (A. V. Delgado-Escueta *et al.*, ed.). Raven Press, New York.]

the lattice, thus allowing for the fact that pyramidal neurons are layered two or more deep in the hippocampal slice.

This general approach to modeling electrical field effects is similar to that described by Traub and co-workers (1985a,b), except that we are now using a larger, and hence more realistic, system (i.e., more cells and more lattice points). We have been able to undertake larger field computations because of certain simplifications in model structure (i.e., no branching dendrites, no dendritic bursting sites) and because we have been able to use the vector facility of the IBM 3090 computer to speed

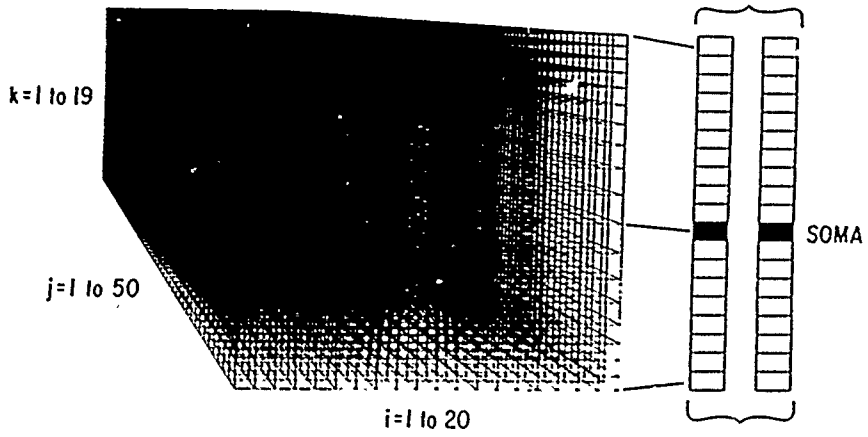

Figure 3.
Schematic diagram indicating structure of computer model. The population of hippocampal neurons consists of 2000 pyramidal cells. A lattice with dimensions 20 × 50 × 19 is used to calculate extracellular potentials. Each neuron in the 20 × 50 population contains 19 compartments, including the soma (shaded). The 19,000 lattice points represent extracellular locations throughout the network of neurons. The lines between each lattice point are extracellular resistors. Each vertical line in the extracellular lattice corresponds to two model pyramidal cells, thus yielding a total of 2000 neurons. The soma of each neuron has active currents, and is attached to two equivalent cylinders (one each for the basilar and apical dendrites). Excitatory synapses connect the pyramidal cells in some versions of the model. A separate population of neurons is excited by pyramidal cells, and in turn inhibits pyramidal cells, thus simulating the recurrent inhibitory circuit.

up the calculations. We briefly note that the differential equations for neuronal membrane behavior are solved using an explicit Taylor series method. The 19,000 linear equations for the extracellular potentials, equivalent to a discrete version of Poisson's differential equation, are solved with an iterative overrelaxation method (Varga, 1962). Other technical considerations concerning methods have been or will be given elsewhere (Traub *et al.*, 1985a,b, 1987).

b. Simulation of Synchronous Bursting with Electrical Interactions. The experimental observations with low-$[Ca^{2+}]$ solutions revealed several specific phenomena (Taylor and Dudek, 1982b; Jefferys and Haas, 1982; Konnerth *et al.*, 1984), and an accurate model would be expected to replicate these electrophysiological characteristics. First, repetitive large population spikes in the field potential recording occurred synchronously with action potentials recorded intracellularly in nearby neurons. Action potentials in a particular cell, however, did not always occur one-to-one

with population spikes, since individual cells sometimes failed to fire during particular population spikes. However, when a cell did fire, it was almost always in phase with a population spike. Furthermore, spike synchrony, or near-synchrony, could occur over large areas (hundreds of micrometers or more). Several experimental observations also indicated that the synchronized firing did not result simply because of perfectly identical kinetics for voltage-dependent conductances across the population. For example, if an individual cell was hyperpolarized by injected current, it rapidly resumed firing in phase with the population upon terminating the current injection (Taylor and Dudek, 1984b). Methods were used in the model to ensure that this type of artifactual synchrony was not present. Finally, the electrical field effect from a population spike yields a somatic transmembrane depolarization during differential recording, whereas intracellular recordings referenced to ground often show a brief negativity in phase with the population spike. These were key features of the electrophysiological data that we felt needed to be present in any accurate simulation of this experimental situation.

In the early simulations of electrical interactions with the 100-cell model, Ca^{2+} currents and Ca^{2+}-dependent currents were blocked (Traub *et al.*, 1985a). The cells were made hyperexcitable by effectively lowering threshold, and spontaneous, repetitive firing was induced with depolarizing currents injected into the somata of 16 cells in the middle of the array. Similar to the data shown in Fig. 4 from a 2000-cell model (see below), it was possible to produce synchronous firing with field effects alone, as long as extracellular resistance was made large enough. These simulations had many of the properties characteristic of the electrophysiological recordings in low-$[Ca^{2+}]$ solutions. For example, transmembrane recordings showed field-effect depolarizations, while single-ended intracellular recordings (i.e., with respect to ground) showed small, brief negativities synchronous with the population spikes. When the extracellular resistance was low, a variety of plausible arrangements of electrotonic junctions did not by themselves cause synchronous firing. When field effects were of intermediate strength, however, electrotonic junctions tended to enhance synchronization. These initial simulations emphasized the importance of electrical field effects and provided a theoretical framework for understanding how they operate. The simulations also suggested that, at least under the conditions studied, electrotonic junctions had relatively little effect on synchronization of the population activity.

Recent preliminary studies on electrical field effects have now been undertaken with a 2000-cell model after some simplifications. Although it is possible to simulate population bursting with field effects alone in this model, the initial studies suggest—surprisingly—that the model is less robust when more cells are present; that is, synchronized firing is only observed under rather specific circumstances. Because of boundary

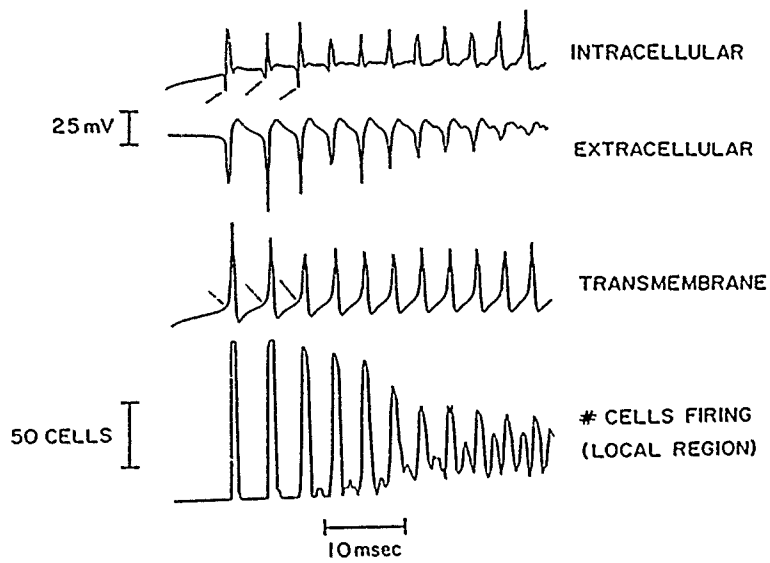

Figure 4.
Computer simulation of synchronous burst in a low-[Ca^{2+}] solution. Intracellular, extracellular (both relative to ground and recorded from the cell body layer), and transmembrane (intracellular minus extracellular) potentials are shown for a typical hippocampal neuron in the model. The bottom trace shows the number of cells that are depolarized > 20 mV in a local region (120 cells) around the illustrated neuron. Chemical synapses and Ca^{2+} conductance are blocked. Note that the intracellularly recorded action potentials are synchronized with the population spikes. The brief negativity preceding each action potential in the single-ended intracellular recording (upper trace, arrows) corresponds to a field-effect depolarization in the differential recording of transmembrane potential (third trace, arrows).

conditions, the extracellular fields are not homogeneous in space and field effects may actually tend to desynchronize spikes in neurons at different locations. Nevertheless, if the intrinsic membrane properties of the cells are similar enough, the basic features of field-induced synchrony (as outlined above) are still observed in a model with 2000 cells (Fig. 4). It is interesting to speculate that electrotonic junctions might compensate for the dispersant tendencies seen in the 2000-cell model, and might act to maintain synchrony.

B. Electrical Field Effects with Chemical Synapses

1. *Differential Recording of Field-Effect Depolarizations*

Although electrical field effects appear to be important for synchronizing neuronal activity in low-[Ca^{2+}] solutions, a critical issue is whether they are effective during convulsant-induced synchronous

bursting, when ionic constituents are normal or nearly normal. Based on the hypothetical mechanism for their occurrence (Fig. 2), one would intuitively expect that the electrical field associated with the population spike would tend to excite inactive cells under any conditions. Differential recording of transmembrane potentials (Fig. 5) during synchronized bursting in the presence of the $GABA_A/Cl^-$ channel-blocker, picrotoxin, combined with intracellular hyperpolarizing current or injection of QX314 to block action potentials in the impaled cell, has clearly revealed field-effect depolarizations on the peak of depolarization shifts (Snow and Dudek, 1984b). Similarly, orthodromic stimulation of afferents to hippocampal pyramidal cells in normal media revealed field-effect depolarizations, and these events could be larger than chemically mediated synaptic potentials under some conditions (Snow and Dudek, 1986). These data raised the issue of how electrical field effects influence the convulsant-induced epileptiform field potential and also how they might operate in the normal brain.

2. *Contribution of Field Effects to the Shape of the Epileptiform Field Potential*

Since large field potential transients are known to occur during epileptiform bursting, we undertook computer simulations of this situation using both recurrent excitation via chemical synapses and also electrical field effects in the model. An analysis of the interactions between chemical synaptic and electrical mechanisms was obtained by combining the two models described above (Traub *et al.*, 1985b). Under these conditions it was possible to simulate the extracellular, intracellular, and transmembrane recordings during synchronous bursting in picrotoxin (Fig. 6). Again, when extracellular resistance was low, the field potential revealed relatively little action potential synchronization. However, clear population spikes were present when extracellular resistance was increased. These initial data supported the hypothesis that chemical synaptic mechanisms synchronized neurons over the time scale of tens and hundreds of milliseconds, whereas electrical field effects synchronized cells in the millisecond time range, thus producing the "teeth" in the "comb-shaped" epileptiform field potential. More recent studies concerning propagation of synchronized bursts in the CA3 area suggest that the properties of local excitatory chemical synapses and axonal conduction, rather than electrical field effects, determine propagation rate (Traub *et al.*, 1987a; Miles *et al.*, in press).

Although these simulations have been able to reproduce experimental observations, a major effort continues to be directed toward increasing the precision of important parameters in the model and toward including different physiological effects. Several types of recently obtained data have been incorporated into the latest versions of the model (Traub *et al.* 1987, 1988, Miles et al., in press), such as the spatial distribution of excitatory synaptic connections, the existence of slow inhib-

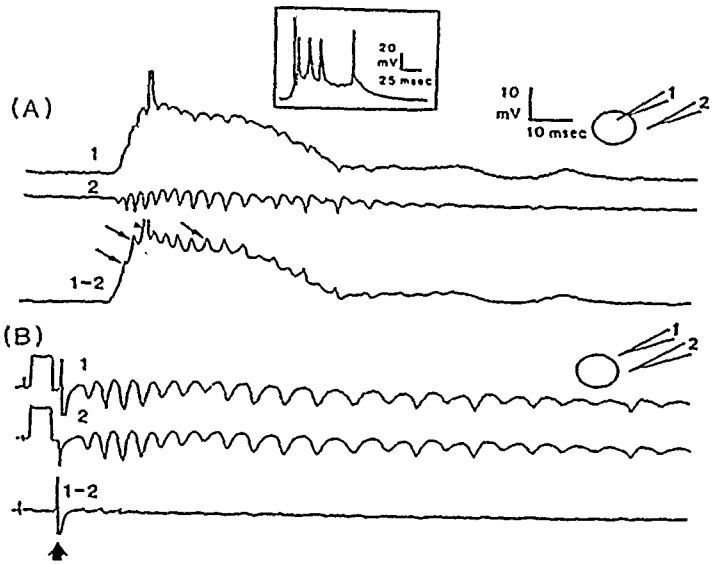

Figure 5.
Differential recording of transmembrane potential during a synchronous burst by pyramidal cells in a picrotoxin-treated hippocampal slice. The enclosed inset shows a spontaneous spike burst at resting potential. (A) Field-effect depolarizations were revealed with differential recording when steady hyperpolarizing current was injected through the intracellular microelectrode to block most of the action potentials in the impaled neuron. The top trace (1) is a single-ended intracellular recording (i.e., with respect to a remote bath ground), the middle trace (2) is an extracellular recording (also with respect to a remote bath ground), and the bottom trace (1 −2) is a recording of transmembrane potential. At resting potential, spikes were synchronous on both electrodes, but slightly inactivated during the large, slow depolarization. In the differential recording (1 −2), field-effect depolarizations (arrows) could be seen during the spike burst when hyperpolarizing current was injected and only one spike (arrowhead) was generated in the impaled neuron. (B) Extracellular control recordings after loss of the impalement. The electrode that had been intracellular (electrode 1, see diagrams) recorded the extracellular field potential immediately outside the neuron in response to orthodromic stimulation (at arrow). Differential recording (1 −2) under these conditions showed that the electrodes had been electrically close. A 10-mV, 5-msec calibration pulse preceded the stimulus. [Reproduced from Dudek *et al.* (1986). Role of Electrical Interactions in Synchronization of Epileptiform Bursts. *In* "Basic Mechanisms of the Epilepsies" (A. V. Delgado-Escueta *et al.*, ed.). Raven Press, New York.]

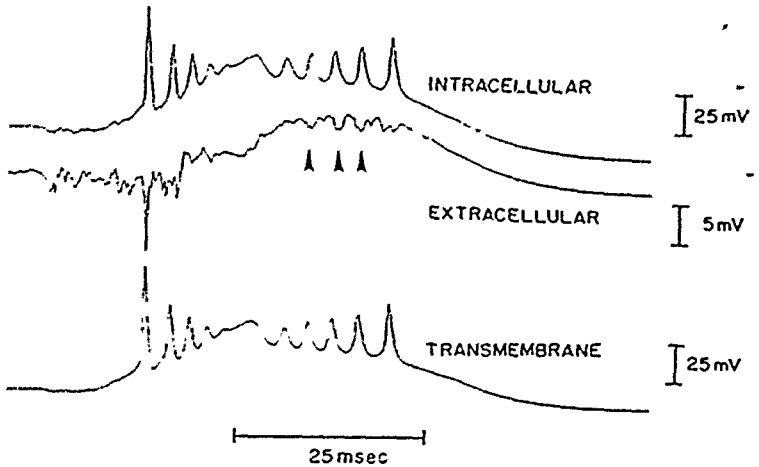

Figure 6.
Simulation of synchronized burst. The model for this simulation had 2000 pyramidal cells with 10 excitatory synaptic inputs per cell. Fast inhibition was blocked, excitatory synapses were functional, and Ca^{2+} conductance and Ca^{2+}-dependent K^+ conductance were present (i.e., conditions corresponding to Fig. 5). Stimulation of a single cell elicited the event. Arrowheads indicate synchronization of action potentials in individual cells with population spikes in the local field potential.

itory postsynaptic potentials (IPSPs) during and after synchronized bursts, and the possibility of developing synchrony when some degree of fast inhibition is present. Careful consideration is now being directed to the magnitude of unitary (Miles and Wong, 1986) and synchronized synaptic currents (Johnston and Brown, 1981), together with anatomical (Boss *et al.*, 1987) and physiological (Miles and Wong, 1986) data on cell density and connection probability. We anticipate that attention to all of these matters will be important for an accurate temporal and spatial synthesis of epileptiform field potentials (Swann *et al.*, 1986). An example of a simulation with 2000 pyramidal cells and slow inhibition from 100 additional neurons is shown in Fig. 6.

III. Discussion

A. Contribution of Local Interactions to Burst Synchronization

1. Chemical Synapses

Several lines of evidence suggest that local excitatory chemical synapses are important for synchronous bursting in hippocampus, but they

that synchronous bursting in the presence of penicillin or picrotoxin is blocked with low-[Ca^{2+}], high-[Mg^{2+}] solutions. Miles *et al.* (1984), moreover, showed that low-[Ca^{2+}], high-[Mg^{2+}] solutions and solutions containing an excitatory amino acid antagonist (γ-D-glutamylglycine or D-2-amino-5 phosphonovalerate) could block synchronous bursting without a significant decrease in membrane excitability. However, several studies have now shown that synchronous bursts of action potentials can also occur in low-[Ca^{2+}] solutions that block chemical synapses (Taylor and Dudek, 1982; Jefferys and Haas, 1982; Konnerth *et al.*, 1984). Different mechanisms pertain in the two conditions. For the condition where synaptic inhibition is blocked and synaptic excitation is still functional, a cascade of neuronal activation appears to be necessary: bursting in a small population of neurons causes an ever-expanding recruitment of other pyramidal cells. Although the available data are limited, the particular susceptibility of the CA3 area for synchronous bursting is likely due to the presence of a larger number or more powerful recurrent excitatory connections in CA3 than in other areas (i.e., CA1 and dentate gyrus). Furthermore, the pronounced intrinsic burst-generating properties of the CA3 pyramidal cells will facilitate transmission of excitatory activity from cell to cell. Two important features of the model are excitatory synaptic connectivity and intrinsic burst generation, which in turn are responsible for burst propagation and synchronization. The computer model with chemical synapses alone not only predicts the previously established observation of a relatively long burst latency to a localized stimulus, but also that intracellular stimulation of a single cell with a depolarizing current pulse should evoke population bursts. Further experimental and modeling work is still required to account quantitatively for burst synchronization and spread in terms of the measured number and strength of excitatory synaptic connections in the hippocampus.

2. *Electrical Field Effects*

The computer models that incorporate only electrical field effects—without chemical synapses—can also generate synchronous population spikes similar in some respects to models with only chemical synapses as a mechanism for local communication. The parameters used in this model are reasonably realistic. Like the models with only chemical synapses, action potentials in active cells must be able to recruit inactive cells or at least influence the timing of their action potentials. This will happen in low-[Ca^{2+}] solutions because inactive cells are close to firing threshold and because inactive cells will "see" fields generated by large numbers of simultaneously firing cells. Simulations with relatively small neuronal networks (i.e., hundreds of cells) indicate that spontaneous activity in a few cells can activate a sufficiently excitable population to fire synchronously. Models with electrical field effects and those with

chemical synapses alone are most likely to fire population bursts if a *group* of synchronously firing cells acts as the initiator of the activity. It is tempting to speculate that these are electrically coupled clusters (see below), but synchronous afferent input from some other structure or the "chance" synchronization of a small group of adjacent cells could also start the cascade of bursting neurons. A single cell thus can activate the whole population if it first excites a small group or cluster of cells, which in turn start the cascade. Presently most modeling and experimental data argue that the primary effect of electrical fields is to synchronize neurons on a fast time scale, thus leading to the relatively smooth waveform often observed for spontaneous population spikes. Slower effects of electrical fields are also possible, but they will require both difficult experiments and computer simulations to test their feasibility (see below).

3. *Electrotonic Coupling*

Evidence for electrotonic coupling via gap junctions in hippocampus and elsewhere in the mammalian brain has long been controversial. Virtually all of the computer modeling has suggested a minor or nonexistent role for electrotonic coupling in the generation, synchronization, and spread of hippocampal bursts. As indicated above, both the model with chemical synapses alone and the one with electrical field effects alone suggest that synchronous activity within a small group or cluster of neurons, such as would be expected of a small coupled network, is a particularly effective means for initiating the synchronization process in a hippocampal network. Several studies in both hippocampus and neocortex have shown significant (if not dramatic) blockage of dye coupling with treatments that cause intracellular acidification (Connors *et al.*, 1984; Gutnick and Lobel-Yaakov, 1983; MacVicar and Jahnsen, 1985). These data argue that gap junctions mediate dye coupling, and for our purposes here they also open up experimental avenues for exploring the importance of coupling to synchronization and spread of epileptiform bursts. Similarly, the data of Gutnick and co-workers (1985) suggest that the slice procedure and associated dendrotomy may increase junctionally mediated coupling in some systems. This observation implies that coupling may be quite sensitive to external influences. On the one hand, a more direct and quantitative assessment of the number of junctions and the strength of coupling is necessary; that is, it is important to know why different methods of assessing coupling give different measures of strength and numbers of coupled cells (see Dudek *et al.*, 1983, 1986, for discussion). It would be valuable to determine whether altering the amount of coupling causes changes in the synchronization of hippocampal activity. To be specific, is it possible to block electrotonic coupling and still obtain synchronous activity? It is somewhat surprising that

electrotonic coupling is classically considered a mechanism for synchronizing electrical activity (e.g., in mammalian inferior olive, and in the pattern-generation networks of invertebrates), but it does not appear to be important for synchronization in hippocampus. This is probably a direct function of the limited amount of coupling thought to be present in cortical structures.

4. *Ionic Changes*

We have not discussed any of the experimental data that involve alterations in extracelluar $[K^+]$ and $[Ca^{2+}]$ during synchronous bursting, and the possible role that these alterations in ionic concentrations might play in synchronizing and recruiting cells. The main reason for this is that ionic shifts have not yet been incorporated into computer models in a direct sense. Recent experimental data argue that increases in extracellular $[K^+]$ are responsible for the slow spread of synchronous bursts in low-$[Ca^{2+}]$ solutions (Konnerth *et al.*, 1986; Yaari *et al.*, 1986). The available data suggest that K^+ acts on a very slow time scale, generally increasing the excitability of the neuronal population and ultimately causing an overall increase in spontaneous firing rates. The propagation rate (1 mm/sec) for this excitability increase is much slower than the propagation velocity of epileptiform events seen in convulsant-treated slices (about 100 mm/sec) (Knowles *et al.*, 1987). It is interesting, however, that increases in extracellular $[K^+]$ can cause synchronized bursts resembling those that occur with picrotoxin, although these shifts in $[K^+]$ merely diminish IPSPs without blocking them entirely (Rutecki *et al.*, 1985; Korn *et al.*, 1987).

5. *Cooperativity*

The issue of cooperativity of mechanisms of local interaction is critical, and this would be particularly difficult to assess with experiments alone. A reasonable approach has been to ask the question, "Which mechanism(s) of local communication can synchronize neurons by itself, and which one is most important?" But in a normally functioning physiological system, more than one system can be operative at a time and the importance of a particular mechanism of neuronal interaction may not be apparent under some conditions. For example, chemical synaptic potentials may be necessary to bring cells close enough to threshold in order for electrical field effects to be significant. In a system with low excitability where membrane potential of most neurons is well below threshold, chemical synaptic mechanisms would be expected to be more important since EPSP amplitude is enhanced when a cell is hyperpolarized and no other activity is occurring. Electrical field effects, at least from action potentials, would be ineffective for cells with low excitability. In another example, as indicated earlier, increasing the amount of coupling in a network could conceivably create a cluster of synchronously firing cells that would be particularly capable of evoking population bursts. The importance of this phenomenon may not be apparent until the population of cells in the model is large enough and the coupled cluster is of appropriate size. With regard to changes in extracellular $[K^+]$, it is conceivable that intense activity and the associated alterations in ionic concentrations could lead to cellular swelling, as well as cellular depolarization, which would then increase the effectiveness of electrical fields as a synchronizing mechanism. One extremely important benefit of computer models is the ability to explore the ramifications of these cooperative interactions quantitatively as a means of aiding experimental interpretations.

B. Local Interactions and Hippocampal Potentiation

Several situations may exist where local neuronal interactions in the hippocampus could be involved in phenomena associated with long-term potentiation (LTP) and other forms of synaptic plasticity. One example involves recurrent excitation. It is known that repetitive stimulation leads to a decrease in GABA-mediated inhibition and that removal of this inhibitory system tends to reveal local excitatory synapses (Miles and Wong, 1987a; Christian and Dudek, 1988). Miles and Wong (1987b) have shown that after repetitive stimulation of afferent input to CA3 pyramidal cells, the strength of interaction between weakly connected neurons can be enhanced. Another possible mechanism for alterations in effective synaptic strength involves electrical interactions and the "dissociation" phenomenon that often occurs in LTP experiments (see Dudek *et al.*, 1988). After LTP, there is often a disproportionate enhancement of the population spike; that is, the population spike is substantially larger for a population excitatory postsynaptic potential (EPSP) of a particular amplitude (e.g., Abraham *et al.*, 1985). Repetitive stimulation is known to cause an increase in extracellular resistance (Dietzel *et al.*, 1980, 1982a,b), and this could in turn enhance electrical field effects, thus increasing population spike amplitude. This mechanism would be particularly effective in those LTP experiments where extracellular recordings are used, and extracellular stimulus intensity is adjusted so that a small population spike is evoked for each stimulus before the tetanizing train. Under these conditions, nearly all cells in the population would be expected to have a large EPSP that is close to (but below) threshold, while only a small fraction of the cells actually fire action potentials to each test stimulus. Repetitive stimulation would then increase the amplitude of the EPSP in some cells and cause them to fire, thus leading to a larger population spike, which would in turn cause a larger field-effect depolarization in the cells that were below threshold. Since the large EPSPs have depo-

larized the neurons *close* to threshold, field-effect depolarizations alone may recruit additional neurons. As more neurons are activated, a larger population spike would occur, and this would in turn recruit additional cells through electrical field effects. It has been argued that this would not explain the decrease in latency that has been observed under these conditions (see Abraham *et al.*, 1985); however, field effects directly depolarize cells, and they could in principle cause a decrease in spike latency. It has also been argued that this mechanism is unlikely because the phenomenon of "dissociation" has not been seen after antidromic activation; however, repetitive antidromic stimulation would not necessarily cause the same alterations in extracellular space and resistance seen with synaptic activation. More importantly, during orthodromic activation of hippocampal neurons, virtually all neurons are close to threshold during the peak of the EPSP at the soma, where spikes are initiated and where field effects are most prominent. During antidromic stimulation, neurons whose axons are not activated by the stimulus are well below threshold, so slight increases in field-effect depolarizations at the soma would generally not recruit additional cells. Therefore, one would not expect this mechanism to be operative during antidromic stimulation, but rather only during orthodromic activation. Unfortunately, experiments to address this issue *directly* will be extremely difficult. The reason is technical in nature. Although one can reveal larger field-effect depolarizations with differential recording during orthodromic activation (Turner *et al.*, 1984), it is extremely difficult to conduct *independent* tests of enhanced excitability (e.g., see Taylor and Dudek, 1984a) under these conditions. Computer simulations, however, might provide a means for evaluating this hypothesis. In essence, it is possible to conduct experiments with the computer model that are impossible or extremely difficult to perform in a physiological system.

C. Electrical Field Effects from Synaptic Potentials

Another situation where electrical field effects could be extremely important, and yet where experimental analyses alone would be difficult and inconclusive, involves population EPSPs and their associated sinks and sources. Virtually all of the studies on electrical field effects have dealt with action potentials. Field effects might also occur when synchronous chemical EPSPs occur in a population of hippocampal neurons. Two situations where these are known to be prominent are during (1) spontaneous epileptiform bursts and (2) certain LTP experiments, where synchronous activation of neuronal populations with electrical stimuli are used. The rationale underlying this hypothetical mechanism is that synchronous activation of excitatory synapses at distal dendritic zones, for example, would cause a current sink in the extracellular space at the level of the active synapses and a source or positivity in surrounding

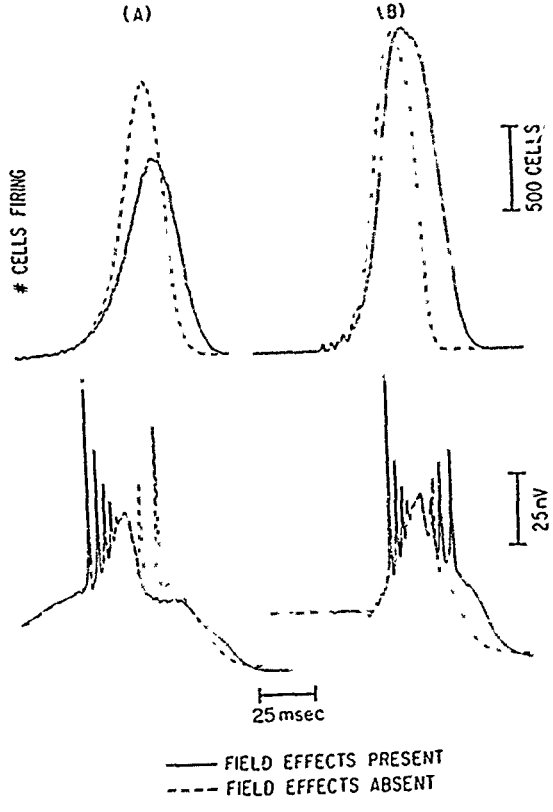

Figure 7.
Preliminary evidence from computer simulations that electrical field effects have complex actions on population bursting. Bursts similar to those shown in Fig. 6 were analyzed either with a low level of inhibition present (A) or with inhibition completely blocked (B). Each cell had 20 excitatory synaptic inputs in (A) and 10 inputs in (B). The number of neurons firing in the 2000-cell model as a function of time is indicated with field effects present (solid line) and absent (dashed line) in the upper set of traces. The intracellular responses of typical cells in the population are shown in the lower traces for each condition. In (A), the slow positivity underlying the epileptiform field potential produces a transmembrane hyperpolarization, which has a net inhibitory effect on the population. In (B), the enhanced generation of action potentials when field effects are present offsets this inhibitory effect.

inactive regions, including the somatic area. In fact, differential recordings during orthodromic activation of hippocampal neurons have shown that EPSPs appear reduced when recorded differentially under these conditions (e.g., compare Fig. 1A1 with Fig. 1A3 in Snow and Dudek, 1986). Unfortunately, it is impossible to prove experimentally that the extracellular somatic positivity arising from distal dentritic input has an inhibitory effect on spike generation. One simply cannot perform real experiments with and without electrical field effects. However, preliminary computer simulations (which were actually aimed at the "dissociation" questioned above) did reveal an inhibitory effect from the population EPSP. When this was incorporated into a model where both recurrent excitation and electrical field effects were present, preliminary simulations indicated that electrical field effects could either enhance or depress synchronous bursting, depending on the details of parameter choices (Fig. 7). A large body of data suggests that synaptic currents substantially contribute to extracellular recordings from large ensembles of neurons (such as the electroencephalogram). Yet, so far, little consideration has been given to the synchronizing or desynchronizing effects of the fields themselves under these conditions. A balance of electrophysiological experimentation and computer simulation will provide new information on this potentially important, but rather difficult, issue.

IV. Conclusion

The aim of this chapter has been to review recent work combining intracellular and extracellular recordings from hippocampal slices with computer simulations of complex neuronal networks. This strategy has revealed numerous insights into the function of neuronal populations that would not have been clear from just one or the other approach alone. The strength of previous modeling research of this nature has been its use of electrophysiological data as the critical input for deriving parameters, and its ability to predict future experimental outcomes. On the other hand, the value of the model for future electrophysiological experimentation will be that tentative priorities can be assigned to certain types of data; that is, some parameters in the model greatly affect the behavior of the system, whereas other parameters appear to have little or no effect. As the model continues to become more detailed, it will allow us to answer questions that are not amenable to experimentation, at least with available techniques. Nonetheless, a constant interplay between electrophysiological experimentation and the quantitative theoretical framework associated with computer models of neuronal networks will be essential for understanding the dynamic behavior of large neuronal ensembles characteristic of the mammalian brain.

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Intrinsic and synaptic mechanisms of hypothalamic neurons studied with slice and explant preparations *

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The use of slice and explant preparations has allowed major advances in our understanding of the membrane physiology of mammalian hypothalamic neurons. This article will review intracellular electrophysiological studies of neurons in or immediately surrounding the supraoptic and paraventricular nuclei. Considerable information is now available on the intrinsic membrane mechanisms that control action potential generation and burst firing in magnocellular neuroendocrine cells (MNCs) within these nuclei. Neurons surrounding the paraventricular nucleus have different electrical properties than the MNCs, including low-threshold Ca^{2+} spikes and pronounced anomalous rectification. Bicuculline and kynurenic acid strongly depress fast IPSPs and EPSPs in MNCs, thus suggesting that inhibitory and excitatory amino acids mediate fast synaptic transmission in the hypothalamus. The effects of neuromodulators, such as noradrenaline and opioid peptides, have also been examined. Noradrenaline excites supraoptic neurons and leads to phasic firing through an alpha-1 mechanism and decreased K^+-conductance. Opioid peptides act directly on μ-receptors to hyperpolarize about half of the neurons through an increased K^+-conductance. In conclusion, using the magnocellular neuroendocrine system as a model, in vitro slice and explant preparations have allowed the characterization of electrophysiological properties, the identification of neurotransmitters for synaptic events, and studies on the mechanism of action of neuromodulators.

Introduction

In spite of considerable interest in the electrophysiology of neuroendocrine cells, intracellular recordings from the mammalian hypothalamus had been rare until recently. Intracellular studies on the mammalian hypothalamus of intact animals have not been feasible, because of its location at the base of the brain and the pulsations caused by the high degree of vascularization in this region. One alternative strategy has involved intracellular recording from neurosecretory cells of invertebrates. Although these preparations have provided the experimental basis for numerous hypothetical mechanisms, phylogenetic differences require that many questions be examined in mammalian preparations. Cultured mammalian neurons have been used to address this issue; however, they are derived from prenatal or early postnatal animals, and their electrophysiological properties may reflect an early stage of development or may be altered by culturing. The use of acute in vitro preparations of slices or explants (Fig. 1) has partially resolved these problems. This article describes recent developments concerning the electrophysiology of hypothalamic neurosecretory cells using these preparations.

Although several neurosecretory systems are present in the hypothalamus, the magnocellular neuroendocrine cells (MNCs) of the paraventricular and supraoptic nuclei (PVN and SON) have

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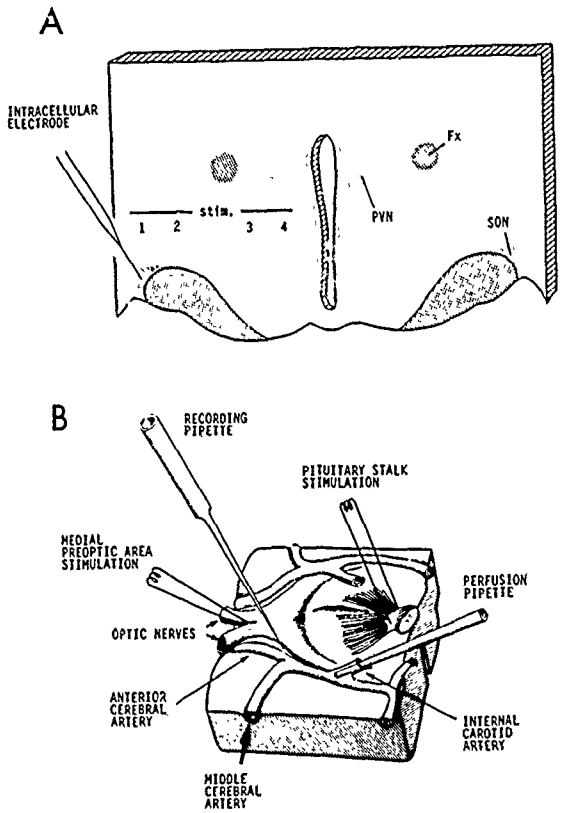

Fig. 1. Schematic diagrams of in vitro hypothalamic preparations. A: coronal hypothalamic slice. Intracellular recordings are obtained from the supraoptic (SON, as shown), paraventricular (PVN) or other hypothalamic nuclei with the slice preparation. Local extracellular stimulation can be applied at several sites around the intracellular recording electrode (stim. 1–4). The optic tracts, third ventricle and fornix (FX) serve as landmarks for positioning the recording and stimulating electrodes (modified from MacVicar et al., 1982). B: basal diencephalic explant. An intracellular recording pipette is positioned in the SON. The pituitary stalk can be stimulated extracellularly to trigger antidromic action potentials, and the medial preoptic area can be stimulated to evoke synaptic responses. A perfusion pipette is shown in the internal carotid artery (from Bourque and Renaud, 1983).

served as a model system for a wide range of neurobiological studies. The PVN and SON contain neurosecretory cells that synthesize vasopressin and oxytocin, which are transported to the neurohypophysis where they are secreted into the general circulation. These hormones are important in numerous physiological functions. Vasopressin is released during hemorrhage and dehydration, and serves the homeostatic function of maintaining proper blood volume and osmolality. Oxytocin is released during lactation and parturition.

Extracellular recordings have yielded a large body of information on the firing patterns of MNCs under physiological conditions (see Poulain and Wakerley, 1982). For example, dehydration and hemorrhage are correlated with enhanced vasopressin secretion and cause phasic firing in about half of the MNCs; this bursting pattern is classically attributed to vasopressinergic MNCs. During lactation, an intense and synchronous burst of action potentials from the other half of the MNCs (i.e., non-phasic MNCs) occurs before milk ejection; these cells are considered to be oxytocinergic. Thus, MNCs of the PVN and SON represent a model neurosecretory system for electrophysiological studies both in the intact animal and also at the level of neuronal membranes and conductances.

Intracellular electrophysiological studies in slices and explants have addressed several important questions concerning the mechanisms underlying the different firing patterns of MNCs: the intrinsic electrophysiological properties, the transmitters responsible for fast PSPs, and the action of neuromodulators. This paper will provide examples of information obtained in each of these areas. Reviews of the electrophysiology of hypothalamic SON and PVN neurosecretory cells are available (Dudek and Andrew, 1985; Renaud et al., 1985; Renaud, 1987).

Methods

Slice and explant preparation

The details for preparing and maintaining slices and explants have been discussed in several of the papers listed in the references. Most studies have been performed on young adult rats, but guinea pigs have also been used. For both preparations, the brain is quickly but gently removed, with special care directed at the cranial nerves (particularly the optic nerves). For the perfused explant preparation, the arteries and neurohypophysis must also be dissected carefully. The hypothalamus is then blocked with a razor blade. To record

from the explant preparation, the block of hypothalamus is pinned to Sylgard, and a perfusion pipette (150–200 μm tip diameter) is inserted into the internal carotid artery. To prepare slices, the block of hypothalamus is cut with a tissue chopper, vibroslicer or vibratome. Slice thickness varies from 400 to 600 μm. The preparations are either kept at a liquid–gas interface or slightly submerged. Most of the recent work in our laboratory has been done using the ramp-type chamber (Haas et al., 1979).

Neuropharmacological and electrophysiological techniques

One important advantage of acute in vitro preparations is the ability to apply drugs and/or alter the ionic concentration of the media relatively rapidly. It is possible to make these experimental manipulations with known concentrations or at specific locations. For rigorous pharmacology and biophysics, it is critical that the precise concentration of drugs and ions be known. This is best done by changing the solution perfused onto the preparation, but one can also inject a small volume of a concentrated solution into the perfusion tubing and then calculate or measure the concentration of substances reaching the preparation. A faster method for testing the effects of pharmacological agents on electrophysiological responses is with microapplication near the recorded cell using iontophoresis or pressure ejection through micropipettes. Although microapplication methods have the important advantage of being more rapid and readily reversible, the drug concentration is generally unknown (i.e. one only knows that the actual concentration at the recorded cell is less than or equal to the concentration in the micropipette). Both of the microapplication methods are subject to other artifacts and appropriate controls are usually necessary.

The methods of electrical stimulation in vitro are similar to those used for in vivo experiments, except one can visually position microelectrodes in precise locations. However, the problems in vitro are also similar to those encountered in vivo. Extracellular electrical pulses stimulate local neurons as well as fibers-of-passage from distant CNS sites. Low-intensity electrical stimuli do *not* provide preferential activation of local neurons versus fibers-of-passage. In fact, brief electrical pulses are more likely to stimulate fibers-of-passage than local cell bodies and dendrites (Ranck, 1975). This problem is more acute in the slice preparation because stimulating electrodes are usually positioned relatively close to the recording site, thereby increasing the likelihood of activating fibers-of-passage.

This article will focus completely on intracellular recording methods. Nearly all intracellular recordings performed in hypothalamic slices have been undertaken with ultrafine micropipettes made on a Brown-Flaming puller. Resistances generally range from 50 to over 200 MΩ. As in other microelectrode applications, lower resistances enhance signal-to-noise ratio and intracellular current passage, and are particularly important for single-electrode voltage-clamping. In our laboratory and in most others, intracellular microelectrodes are advanced with a microdrive, usually the piezo-electric type, but hydraulic ones can also be used. Impalements are usually obtained by oscillating the negative capacitance feedback of the amplifier. High-quality impalements are indicated by resting potentials greater than −60 mV, action potentials greater than 70 mV, input resistances over 100 MΩ and often by repetitive firing to a depolarizing current pulse. Intracellular staining experiments using these methods have indicated that all impaled neurons in the SON and most in the PVN are magnocellular.

Results

Intrinsic electrophysiology

Although neurons in many areas of the brain fire burst discharges, this pattern of activity has attracted considerable attention in the magnocellular neuroendocrine system because it is known that bursting enhances hormone secretion. A variety of intracellular studies from neurosecretory systems of invertebrate animals and from neurons in other areas of the vertebrate brain, combined with results obtained from extracellular recordings in the magnocellular neuroendocrine system, suggested that phasic firing by vasopressinergic neu-

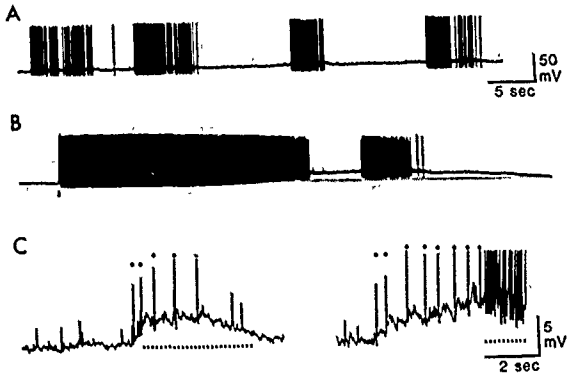

Fig. 2. Intrinsic bursting in supraoptic neurons. A: spontaneous bursts. Four distinct bursts, each lasting several seconds, are shown for an SON neuron. B: evoked afterdischarge. At the arrow, a 100 ms suprathreshold current pulse evoked a spike train, which was followed by a long afterdischarge lasting tens of seconds. Calibrations for A apply to B also. C: summation of depolarizing afterpotentials. High-gain, low-pass-filtered recordings illustrate the depolarizing afterpotentials that followed individual action potentials (closed circles); these afterpotentials could show summation during brief (left) or long (right) bursts (from Andrew and Dudek, 1984a).

rons depends on intrinsic conductance mechanisms. For example, Dreifuss and co-workers (1976) clearly showed with extracellular recordings in vivo that antidromic stimulation from the neurohypophysis could trigger long afterdischarges in putative vasopressinergic neurons. Phasic bursting was recorded in hypothalamic slice and explant preparations (Fig. 2A), indicating that the intrinsic and local synaptic properties of hypothalamic neurons could generate the burst discharges previously seen in intact animals. Consistent with in vivo experiments (Dreifuss et al., 1976), intracellular recordings in the slice preparation showed that a brief depolarizing current pulse in a single MNC could evoke a long afterdischarge of action potentials (Fig. 2B). Thus, a brief period of spike discharge could activate a regenerative mechanism that led to further firing. High-gain intracellular recordings during spontaneous bursts showed that each action potential was followed by a depolarizing afterpotential, which lasted about a second (Fig. 2C); summation of depolarizing afterpotentials during repetitive spikes promoted burst discharges (Andrew and Dudek, 1983, 1984a; Andrew, 1987a). Voltage–clamp studies revealed a postspike aftercurrent (Fig. 3B); this slow inward current was Ca^{2+}-dependent and tetrodotoxin-resistant (Bourque, 1986). Current–voltage relations indicated that this aftercurrent imparts a negative slope resistance near spike threshold (Fig. 3C). Although MNCs have intrinsic mechanisms capable of generating burst discharges, some of the bursts recorded in hypothalamic slices may be driven by synaptic input, presumably from local neurons near the magnocellular nuclei (Andrew and Dudek, 1984a; Andrew, 1987b). Intracellular recording and staining followed by immunocytochemical identification of cells stained with Lucifer yellow revealed that spontaneously phasic MNCs were vasopressinergic (Cobbett et al., 1986). Thus, intracellular electrophysiological studies using slice and explant preparations have provided fundamental information about the mechanism of bursting of MNCs.

Although further studies on the possible electrophysiological differences of vasopressinergic and oxytocinergic MNCs are necessary, a general picture has emerged concerning the electrical properties of MNCs, particularly in the SON. It is known, for example, that both voltage-dependent Na^+ and Ca^{2+} currents contribute to the action potential of MNCs (Andrew and Dudek, 1984a; Bourque and Renaud, 1985a). Repetitive firing of action potentials in all MNCs is associated with spike broadening (Andrew and Dudek, 1985; Bourque and Renaud, 1985b) and followed by an afterhyperpolarization, which is due primarily to a Ca^{2+}-activated K^+-conductance (Andrew and Dudek, 1984b; Bourque, Randle and Renaud, 1985). Bourque (1988) has described a transient outward current, which probably plays an important role in controlling the firing pattern of MNCs. Recently, intracellular recordings from neurons surrounding the PVN have revealed electrophysiological properties that differ considerably from MNCs (Poulain and Carette, 1987; Tasker and Dudek, 1987). In particular, hyperpolarization of these neurons with steady injected currents revealed low-threshold Ca^{2+} spikes, which were not seen in MNCs (Fig. 4A, B). These events were resistant to tetrodotoxin, and could be blocked by Cd^{2+}; they were also much larger and faster than the depolarizing afterpotentials ob-

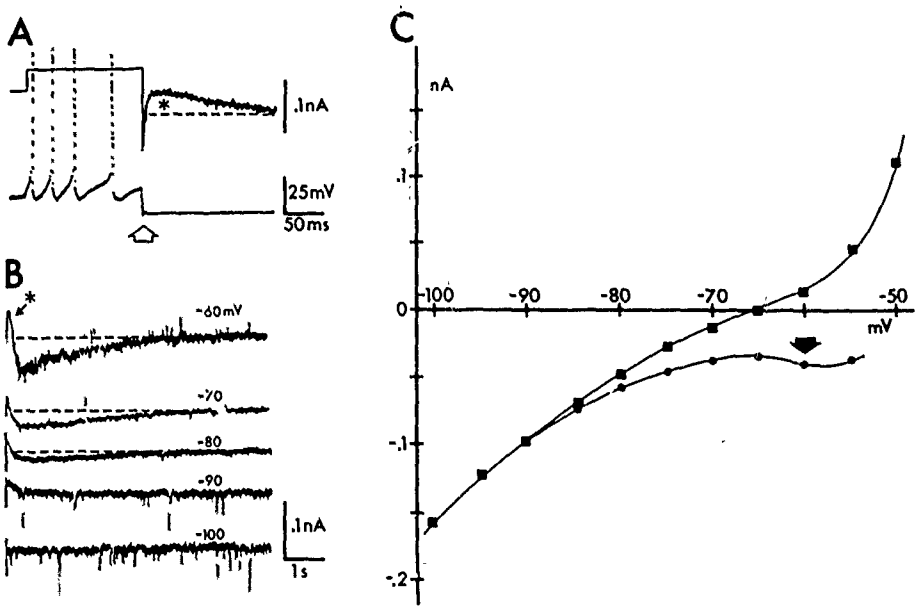

Fig. 3. Single-electrode voltage-clamp recordings of spike aftercurrents in MNCs. A: transient, outward aftercurrent. While membrane potential was held near −70 mV with steady injected current, a depolarizing current pulse (upper trace) was injected into the MNC to evoke action potentials (lower trace). At termination of the current pulse (arrow), the MNC was voltage-clamped to −80 mV. This revealed an early outward current (*), which is normally responsible for the afterhyperpolarization. B: slow inward aftercurrent. The same procedure as in A was used to study the aftercurrent responsible for the depolarizing afterpotentials (note the slower time scale). Aftercurrents were analyzed at several different potentials, as indicated in the figure. The early outward current (*) preceded the slower inward current, which was largest at −60 mV and undetectable at potentials negative of −90 mV. C: current–voltage relations. The amplitude of the current flowing 10 s after the beginning of the clamp indicated the steady-state current–voltage relation (■). The current flowing 500 ms after onset of the clamp was used as a measure of the inward aftercurrent (●). Spike threshold for this MNC was −60 mV (arrow), which is near the region of negative resistance in the current–voltage relation (from Bourque, 1986).

served in MNCs. In addition, the cells with low-threshold Ca^{2+} spikes also showed pronounced anomalous rectification, whereas MNCs had relatively linear current–voltage curves to approximately −90 mV (Fig. 4C, D). Immunocytochemical studies on cells injected with Lucifer yellow suggest that the neurons with low-threshold Ca^{2+} spikes were not MNCs, since they were unstained with antisera to neurophysin (Poulain and Carette, 1987; Hoffman et al., 1988). Therefore, neurons around the PVN (and possibly the SON) appear to have distinctly different electrophysiological properties than MNCs.

Transmitters

Practically every neuroactive substance has been found in the hypothalamus, and there have been numerous hypotheses about how different substances regulate the electrical activity of MNCs and secretion of oxytocin and vasopressin. Anatomical and pharmacological studies have provided indirect evidence concerning the possible function of different substances, although very little *direct* evidence had existed until recently concerning the transmitters that mediate fast synaptic potentials in the hypothalamus. In both slice and explant preparations, synaptic potentials lasting tens of milliseconds are readily observed spontaneously and after extracellular stimulation. A particularly effective approach to the identification of transmitter substances is bath-application of known concentrations of specific transmitter antagonists to evaluate their effects on synaptic potentials. This line of investigation has provided new information about the transmitters that regulate the electrical activity of MNCs.

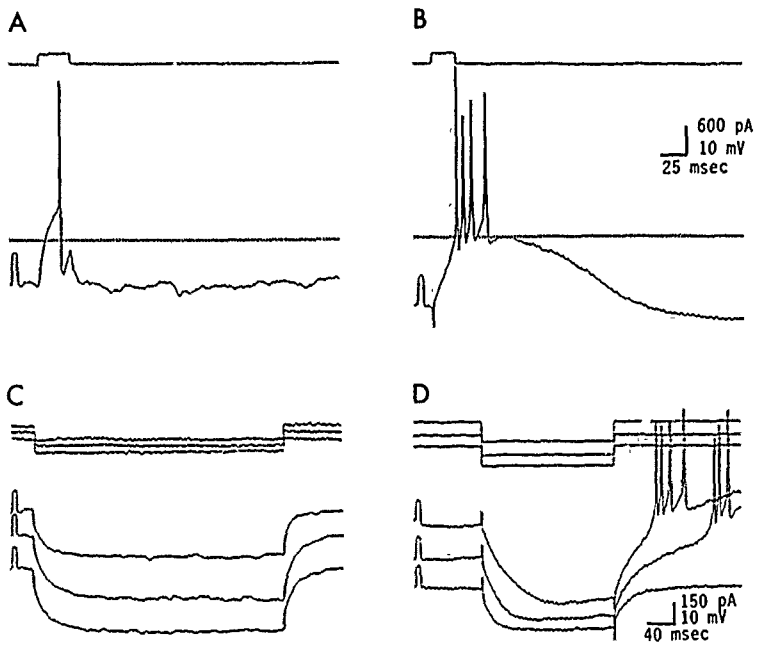

Fig. 4. Low-threshold calcium spikes (LTS). A: putative MNC. A PVN cell with the electrical properties of an MNC did not generate an LTS potential when depolarized (upper trace) from a hyperpolarized membrane potential. B: LTS neuron. A cell located just outside the PVN showed a burst of action potentials superimposed on an LTS potential when the cell was depolarized (upper trace) from a hyperpolarized membrane potential. Dotted lines in A and B represent resting membrane potential. C: current–voltage relations characteristic of MNCs. Hyperpolarizing current pulses (100 pA) were injected at resting membrane potential and increasingly negative potentials in a putative MNC. There was no decrease in input resistance (but sometimes a slight increase) at hyperpolarized membrane potentials. D: anomalous rectification in LTS neurons. Hyperpolarizing current pulses (150 pA) injected at resting potential and at hyperpolarized membrane potentials revealed a profound change in input resistance in an LTS neuron. Decreased input resistance at hyperpolarized levels was indicative of anomalous rectification. Calibration bars in B apply to A and B, and those in D apply to C and D (from Tasker and Dudek, 1987).

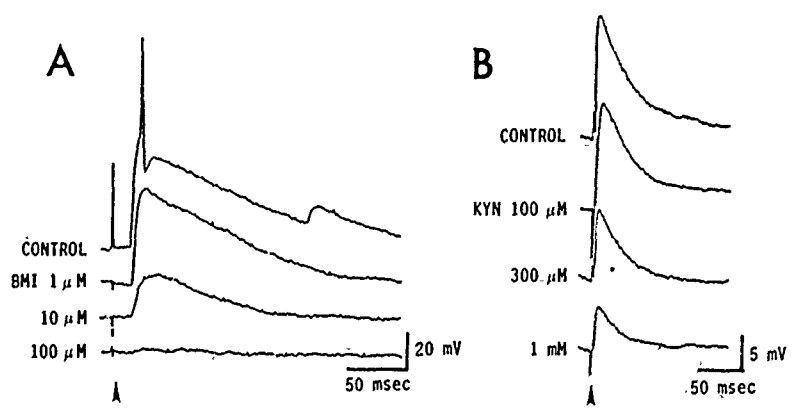

Fig. 5. Effect of antagonists for inhibitory and excitatory amino acid receptors on PSPs of SON and PVN neurons. A: effect of bicuculline on reversed IPSPs. Intracellular recordings were obtained with a KCl-filled electrode. Increasing concentrations of bicuculline methiodide (BMI, 1–100 μM) progressively reduced the amplitude of the reversed IPSPs that were evoked with electrical stimulation of the diagonal band of Broca (from Randle et al., 1986b). B: effect of kynurenic acid on EPSPs in PVN evoked with local electrical stimulation. Intracellular recordings were obtained from PVN neurons in the presence of 50 μM picrotoxin, which blocked all IPSPs. The EPSPs were evoked by extracellular stimuli delivered near the fornix and were superimposed on a hyperpolarizing current pulse (not shown). Increasing concentrations of kynurenic acid (100 μM–1 mM) progressively reduced the amplitude of the EPSPs (from Wuarin and Dudek, 1988).

Both immunocytochemical and pharmacological studies have suggested that γ-aminobutyric acid (GABA) is a major inhibitory transmitter in the hypothalamus, including the magnocellular neuroendocrine system. Recently, Randle et al. (1986b) showed that bath-application of bicuculline blocked spontaneous and evoked IPSPs in supraoptic neurons. These IPSPs were reversed when the cell was hyperpolarized to −72 mV with potassium acetate electrodes, and they had reversal potentials positive of resting potential when recorded with KCl electrodes. Bath application of 10 μM bicuculline attenuated the reversed IPSPs, and 100 μM bicuculline abolished them (Fig. 5A). Comparable effects were also observed with spontaneous IPSPs. Using picrotoxin, we have obtained similar data in slices of SON and PVN (Wuarin and Dudek, unpublished observations). These results argue that GABA is an important mediator of fast IPSPs in the magnocellular neuroendocrine system.

A wide variety of studies in mammalian central neurons, particularly in hippocampus and neocortex, have argued that excitatory amino acids are the primary class of substance mediating fast excitatory transmission in the mammalian brain. Both spontaneous EPSPs and EPSPs evoked by local stimulation dorsolateral to the SON are greatly attenuated by bath-application of kynurenic acid, a broad-spectrum amino acid antagonist (Gribkoff and Dudek, 1988). Recent studies with lower concentrations of kynurenic acid (Fig. 5B) have corroborated and extended this finding in the PVN (Wuarin and Dudek, 1988). These data support the hypothesis that the major excitatory transmitter mediating fast synaptic events in both the SON and PVN is glutamate or a closely related excitatory amino acid.

Neuromodulators

Many other substances are thought to provide chemical regulatory input to the SON and PVN and are known to modify the electrical activity of MNCs. None of the available data on these other substances, however, provide good evidence that they mediate the fast synaptic potentials recorded in all of the types of hypothalamic neurons so far studied. It therefore seems likely that the action of these other substances is relatively slow (on the order of hundreds of milliseconds or longer), and they are likely to have more subtle effects on neuronal membrane properties than the previously described amino acid transmitters. Until further studies are performed using specific antagonists of these substances, they should be considered separately as putative neuromodulators or slow transmitters. Slow depolarizations from single and repetitive stimuli have been observed in SON neurons (Dudek and Gribkoff, 1987), but nearly all of the data are also consistent with activity-evoked changes in extracellular $[K^+]$. Again, rigorous evidence for a slow synaptic potential will require blockade by a specific antagonist at reasonable concentrations, which has not yet been accomplished in the hypothalamus (but see MacVicar and Pittman, 1986). Two examples of possible substances that appear to have quite different properties than those expected of a fast transmitter are noradrenaline and opioid peptides; their actions on MNCs will be briefly discussed below.

Noradrenaline is a particularly important regulator of the magnocellular neuroendocrine system. Bath application of noradrenaline or the α_1-agonist, phenylephrine, depolarized MNCs in SON (Fig. 6A, B) and led to phasic firing (Randle et al., 1986a). Prazosin blocked this depolarization, thus suggesting mediation by an α_1-receptor mechanism (Fig. 6C). The depolarizations caused by noradrenaline were not associated with a change in membrane resistance, and both hyperpolarizing current injection and increases in extracellular $[K^+]$ reduced them. Noradrenaline decreased the duration of spike hyperpolarizing afterpotentials and enhanced the amplitude of the slow depolarizing afterpotentials. These effects suggest that noradrenaline decreases a K^+-conductance. Randle and coworkers (1986a) have suggested that noradrenaline inactivates a transient K^+-current (i.e. 'A-current').

Another type of slow modulatory action on MNCs involves the effects of opioid peptides, which are known to be extensive throughout the hypothalamus. Pharmacological studies in intact animals suggest that opioid peptides depress certain physiological functions thought to be mediated by the magnocellular neuroendocrine system,

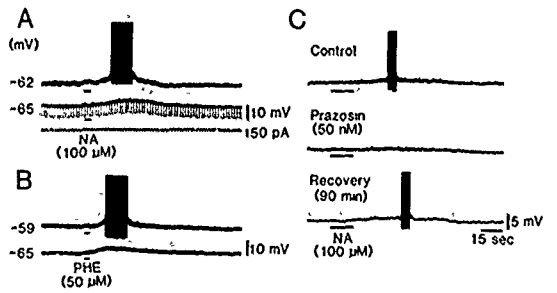

Fig. 6. Effects of noradrenaline on SON neurons. A: action of noradrenaline (NA). The two upper traces are intracellular recordings during two separate applications of NA (100 µM, horizontal bar). At resting potential (−62 mV, upper trace), NA caused a depolarization with superimposed action potentials. When the cell was hyperpolarized with steady injected current (−65 mV, second trace), a clear depolarization was revealed. Input resistance was evaluated with constant current pulses (bottom trace), but NA caused no changes. B: effect of phenylephrine (PHE, 50 µM, horizontal bar) on a different SON neuron. C: effect of prazosin (50 µM) on the NA-induced depolarization and spikes. Upper trace illustrates the effect of NA (100 µM) under control conditions, and the middle trace shows that the actions of NA were blocked by prazosin. After 90 min of recovery, a response to NA could be obtained again (from Randle et al., 1986a).

such as lactation. One likely site of action of opioid peptides is the neurohypophysial terminals (e.g. see Bicknell, 1985, for review). However, extracellular studies in PVN and SON using hypothalamic slices suggested that opioid peptides depress electrical activity at the soma in approximately half of these neurons (Muehlethaler et al., 1980; Pittman et al., 1980; Wakerley et al., 1983).

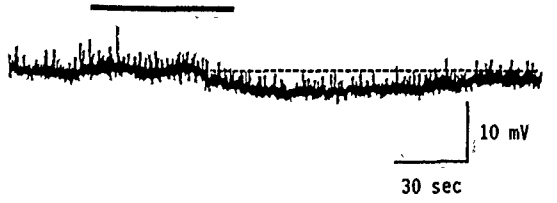

Fig. 7. The effect of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin, a µ-receptor agonist, on a rat SON neuron. Intracellular recordings were obtained in the presence of tetrodotoxin, which blocked all action potentials. The electrodes contained KCl, and thus the Cl^- gradient and the IPSPs were reversed. This was confirmed with bath-application of picrotoxin, which attenuated the reversed IPSPs. Bath-application of the µ-receptor agonist (bar, 10^{-6} M) hyperpolarized the neuron (from Wuarin and Dudek, 1987).

Wuarin and co-workers (1988) have shown that the µ-receptor agonist, [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin, causes a dose-dependent (10^{-8} to 10^{-6} M) hyperpolarization of about one-half of the intracellularly recorded PVN neurons. Naloxone (10^{-6} M) reversibly blocked this effect. Bath-application of a selective δ-receptor agonist ([D-Pen^2,Pen^5]enkephalin at 10^{-6} M) or a selective κ-receptor agonist (U-50,488 at 10^{-6} M) did not hyperpolarize those PVN neurons that were responsive to the same dose of the µ-receptor agonist. On-going studies in our laboratory with SON and PVN neurons from both rat and guinea pig have indicated that this µ-receptor agonist hyperpolarizes some neurons even in the presence of tetrodotoxin, which blocks axonal conduction and spike-mediated synaptic transmission; therefore, µ-receptor agonists may act directly on SON and PVN neurons. This hyperpolarizing effect was also obtained with KCl electrodes, which reversed the IPSPs. Since some drug-induced hyperpolarizations were associated with a decrease in input resistance, µ-receptor agonists probably increase K^+-conductance of these neurons (Wuarin and Dudek, 1987). However, µ-receptor agonists may also inhibit neurons in PVN and SON through presynaptic mechanisms, possibly by depressing the activity of local neurons around the magnocellular nuclei (Wuarin and Dudek, unpublished observations). These studies have begun to provide detailed information about the mechanism of action of opioid peptides in this model hypothalamic system.

Discussion

Similar to acute in vitro preparations from other areas of the mammalian brain, hypothalamic slices and explants allow experiments that are generally not feasible in the intact animal. In both slice and explant preparations, the approximate location of electrophysiological recording, electrical stimulation and drug microapplication can be ascertained during the experiment (and subsequently confirmed more rigorously with various marking techniques). It has been possible to obtain stable intracellular recordings for many hours, while alter-

ations have been made in the ionic constituents of the media and pharmacological agonists and antagonists have been applied to neurons at known concentrations. All of the studies described here required high-quality intracellular recordings so that stable electrophysiological responses could be examined under several conditions. For those experiments using antagonists that block ionic channels or transmitter receptors, the specificity of the effects is concentration dependent. Similarly, application of agonists in the perfusate at known drug concentrations allows one to obtain accurate dose–response curves. Another advantage of bath application is increased confidence that all cells are affected equally, thus reducing false–negative data. Where desensitization may be a problem, visually guided microapplication techniques can be used with both of these in vitro preparations, although the actual drug concentration at the recorded cells is unknown with these techniques. During bath application, all of the presynaptic cells that impinge on the recorded neuron are exposed to the drug. Microapplication can reduce this problem. Blockade of action potentials or chemical synaptic transmission with tetrodotoxin or calcium-channel blockers, respectively, can eliminate presynaptic effects.

The explant and slice preparations each have their advantages. Perfusion of the explant preparation through the internal carotid artery (Bourque and Renaud, 1983) allows the use of a larger block of tissue with more hypothalamic structures; for those nuclei that are present in the explant (e.g. SON), more dendritic processes presumably remain intact. The neurohypophysis can be retained with the hypothalamic explant, thus allowing correlative studies on electrophysiology and hormone secretion to be performed in vitro (e.g. Renaud, 1987). Slices, on the other hand, can be cut in a variety of orientations, thus allowing several dorsally located hypothalamic nuclei (e.g., PVN) to be studied electrophysiologically. Visualization of nuclei and fiber tracts is also facilitated with the slice preparation (see below).

One important problem with both the hypothalamic slice and explant preparations, and with all similar in vitro preparations from other areas of the brain, is that interpretation of experiments using electrical stimulation is difficult. Most inputs have been cut during the preparation, so the cells and/or fibers that are activated by electrical stimulation cannot be identified. Although similar complications are present in vivo (activation of fibers-of-passage is often not given appropriate consideration), the problem is much greater in vitro because of the closer proximity of the stimulating electrode to the recorded neuron. It is essentially impossible to activate local neurons independent of fibers-of-passage with electrical pulses. This technical problem has led to several misinterpretations in the literature.

We have reviewed several examples of recent advances that were made using hypothalamic slices or explants. Unfortunately, it was not possible to cover some of the other important issues that have been studied recently (e.g. osmosensitivity). In the future, several new strategies are likely to be coupled with slice and explant preparations to provide further understanding of hypothalamic electrophysiology. One can expect that additional studies will be undertaken using the single-electrode voltage-clamp to analyze ionic conductances (e.g. Bourque, 1986, 1988) or glutamate microstimulation to activate local neurons independent of axons-of-passage (Tasker and Dudek, 1988). Recorded neurons will be systematically injected with intracellular markers and immunocytochemically processed to correlate electrophysiological properties with transmitter or hormone content. Thinner slices (i.e. less than 300 μm), combined with on-line video- and computer-enhanced image processing techniques, may allow better positioning of electrodes during experiments. In addition, with the use of much thinner slices or isolated neurons dissociated from hypothalamic slices, it may be possible to undertake more rigorous biophysical studies with patch-clamp techniques.

Finally, we should address the question of how intracellular electrophysiological studies conducted in slice and explant preparations have contributed to our understanding of the neuronal control of vasopressin and oxytocin secretion. In vitro experiments alone cannot answer questions about systems physiology at an organismic level; however, when they are designed in concert with in vivo observations they can provide fundamental

information on cellular mechanisms. For example, intracellular electrophysiological studies on LTS neurons thought to occur around and possibly in the magnocellular nuclei now suggest a way to differentiate the electrical activity of neurons during single-unit studies in vivo. It should be possible to devise ways to identify LTS discharges with purely extracellular techniques, and thereby determine their possible role in the regulation of neurohypophyseal hormone secretion.

Several lines of evidence argue that the phasic bursts of vasopressinergic neurons need not be driven by periodic synaptic input. Instead, a tonic increase in the frequency of fast EPSPs or other steady excitatory influences can conceivably depolarize vasopressinergic neurons into a potential range where sequential alterations in voltage-dependent conductances lead to phasic firing through intrinsic mechanisms. Previous confusion about the noradrenergic innervation of the SON and PVN may be reconciled by recent in vitro studies on the mechanisms of action of noradrenaline at the membrane level; its excitatory effect not only involves membrane depolarization, but possibly also a voltage-dependent conductance that would regulate phasic firing. These ideas, in turn, can account for the observation in vivo that the phasic bursts of different vasopressinergic neurons are asynchronous (Poulain and Wakerley, 1982), since one would expect slight differences between neurons to cause oscillations that are out of phase with each other. The oxytocinergic system, on the other hand, is known to fire synchronous bursts of action potentials and the bursts are roughly time-locked across all four nuclei (i.e. both pairs of PVNs and SONs). Thus, synchronous chemical synaptic input is likely to be critical for evoking these bursts. In vitro studies suggest the untested hypothesis that excitatory amino acids are the transmitter responsible for triggering the synchronous bursts characteristic of oxytocinergic neurons before each milk ejection in vivo. These bursts are much more intense than those of the vasopressinergic neurons, and are thought to often be associated with depolarization-induced spike inactivation. Although several substances have been shown to excite MNCs, none of them are as consistent and potent as the excitatory amino acids. Both of these hypothetical descriptions of the neuronal mechanisms responsible for activation of the vasopressinergic and oxytocinergic systems depended first on in vivo experiments and then, more recently, on in vitro studies. Future research with both types of methods will be necessary to test these and other hypotheses about the neuronal mechanisms that control secretion of hypothalamic hormones.

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Effects of Excitatory Amino Acid Antagonists on Synaptic Responses of Supraoptic Neurons in Slices of Rat Hypothalamus

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SUMMARY AND CONCLUSIONS

1. Intracellular recordings from magnocellular neurons in the supraoptic nucleus (SON) were obtained from rat hypothalamic slices to determine the effects of specific transmitter antagonists on evoked postsynaptic potentials (PSPs), action potential afterdischarge, and spontaneously occurring PSPs.

2. Broad-spectrum excitatory amino acid (EAA) antagonists, kynurenic acid (KYN) and γ-d-glutamylglycine (DGG), significantly diminished or eliminated electrically evoked depolarizing PSPs and spike discharges. These compounds also greatly reduced the amplitude and frequency of spontaneous PSPs.

3. The specific *N*-methyl-D-aspartate (NMDA) receptor antagonist, DL-2-amino-5-phosphonopentanoic acid (AP5), did not significantly reduce these measures of synaptic activation under these experimental conditions.

4 The γ-aminobutyric acid (GABA) antagonist, bicuculline methiodide (BIC), partially antagonized some PSPs when the cells were hyperpolarized (−75 to −80 mV) with steady injected currents; KYN antagonized BIC-resistant PSPs.

5 The involvement of a hypothetical cholinergic input to the SON in the responses to stimulation of the region dorsolateral to the SON was tested by bath application of nicotinic cholinergic antagonists, particularly *d*-tubocurarine (dTC). Nicotinic cholinergic antagonists, even after prolonged exposure to high concentrations, did not block the responses of SON cells to dorsolateral stimulation.

6 These findings strongly suggest that EAAs mediate fast excitatory synaptic responses of SON neurons to stimulation of cells and axons in the region dorsolateral to the SON. The blockade of almost all spontaneous EPSPs by broad-spectrum EAA antagonists likewise argues that EAAs are responsible for the majority of ongoing fast excitatory input. These responses appear to involve an interaction with kainate- and/or quisqualate-type EAA receptors.

INTRODUCTION

The magnocellular neuroendocrine system of the mammalian hypothalamus, which includes the neurons of the supraoptic nuclei (SON), is responsible for the release of the peptide hormones oxytocin and vasopressin from the neurohypophysis (Ivell et al. 1983; Silverman and Zimmerman 1983; Swanson and Sawchenko 1983). Several factors have contributed to its status as a model neuroendocrine system in mammals. These include the well-characterized cause-effect relationship among extrinsic stimuli, hormone release, and physiological responses. Examples are the relationship between increased blood osmolality and vasopressin release (Poulain and Wakerley 1982, Sladek and Armstrong 1985) and the involvement of oxytocin in the milk-ejection reflex (Lincoln and Wakerley 1974; Robinson 1986). Also, the accessibility of magnocellular neuroendocrine cells for electrophysiological studies has led to considerable progress in determining the responses of these cells to physiological stimuli and in identifying the intrinsic cell properties that contribute to these responses (for reviews, see Poulain and Wakerley 1982; Renaud et al. 1985, 1987). Until recently, however, very little was known in this system about the synaptic mechanisms that are responsible for translation of extrinsic physiological stimuli into appropriate responses of the magnocellular neurons.

Recent anatomic and physiological studies have focused on γ-aminobutyric acid (GABA) as the mediator of inhibitory synaptic responses, and this work has established GABA as an important transmitter in the SON (Jhamandas and Renaud 1986, Randle et al. 1986, 1987; Theodosis et al. 1986; Van den Pol 1985). Although anatomic, hormone-release, and preliminary physiological evidence have suggested roles for several excitatory transmitter candidates in the SON (Arnauld et al. 1983; Bioulac et al. 1978; Hatton et al. 1983), there has been a lack of direct electrophysiological evidence linking a particular transmitter substance to individual synaptic events. In a previous brief report (Gribkoff and Dudek 1988), we presented preliminary evidence that kynurenic acid (KYN), a specific broad-spectrum antagonist of excitatory amino acid (EAA) receptors, reduced or abolished components of the synaptically evoked response to extracellular electrical stimulation dorsolateral to SON. It is now widely believed that EAAs are the most important and widespread class of excitatory transmitters in the mammalian central nervous system. Our observations here with EAA antagonists provide strong evidence that EAAs are extremely important in the generation of fast synaptic potentials of the magnocellular neuroendocrine system in rat hypothalamus.

In the present study, we have further tested the hypothesis that an EAA is the primary mediator of excitatory synaptic responses evoked by electrical stimulation dorsolateral to the SON. This included a more quantitative evaluation of the effects of KYN, assessment of the effects of γ-d-glutamylglycine (DGG, another broad-spectrum EAA antagonist), and the determination of actions of the specific *N*-methyl-D-aspartate receptor (NMDA) antagonist 2-amino 5-phosphonopentanoic acid (AP5) on SON neuronal responses to stimulation of the region dorsolateral to the SON. In addition, the effects of a GABA antagonist and antagonists of nicotinic cholinergic receptors on these synaptic responses were also assessed.

METHODS

Coronal hypothalamic slices at the level of the optic chiasm (400–600 μm) were obtained from young rats (120–190 g) of either sex by the use of techniques described in detail elsewhere (Dudek and Gribkoff 1987). The slices were transferred to a recording chamber (Haas et al. 1979) and maintained at 32–34°C in the presence of warmed, oxygenated medium. The composition of the control incubation medium was (in mM) 124 NaCl, 26 $NaHCO_3$, 3 KCl, 1.3 $MgSO_4$, 2.5 NaH_2PO_4, 2.4 $CaCl_2$, and 10 glucose. The pH of all solutions was ~7.4. The solutions were delivered by a peristaltic pump at a constant rate of 1.5 ml/min. All drugs were freshly dissolved in normal medium and applied by bath perfusion.

Neurons were impaled in the SON with glass microelectrodes filled with 3 M K^+-acetate (100–200 MΩ). Hypothalamic slices were transilluminated, and recording electrodes were visually guided into the SON. Estimates of input resistance were made by measuring the voltage deflection resulting from the injection of hyperpolarizing current pulses (80–100 ms, −100 pA) through a balanced bridge circuit; cells were often current clamped at a hyperpolarized or depolarized membrane potential by intracellular current injection. Bridge balance was constantly monitored and adjusted when necessary. Resting membrane potential was estimated at the time of cell penetration and verified, when possible, at the termination of the recording. Intracellular recordings were obtained with the use of a standard intracellular microelectrode amplifier (Neurodata); signals were stored on magnetic tape for later analysis. Cells included in the tabulation of results had a resting membrane potential greater than or equal to −55 mV and input resistance ≥100 MΩ; recordings from neurons included in this study typically exceeded 1 h and lasted as long as 4 h.

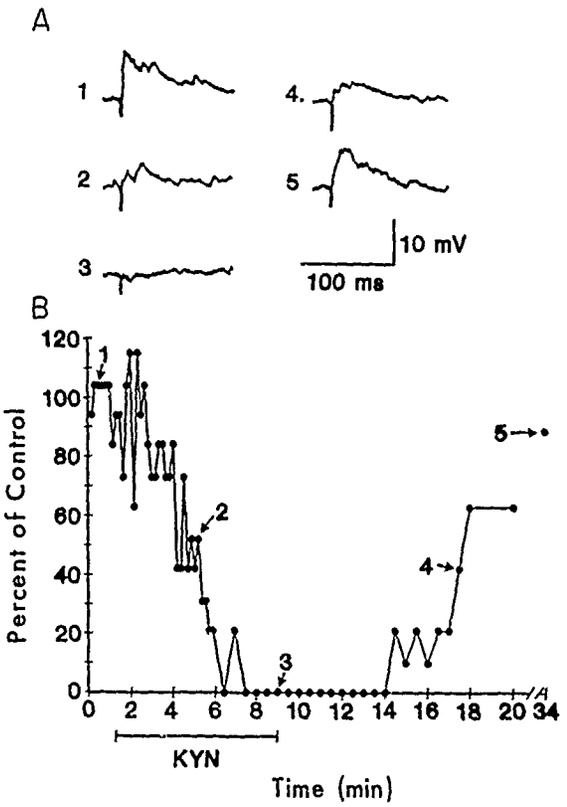

FIG. 1. Time course of the antagonism of the evoked PSP by KYN (2.0 mM). *A*: evoked PSPs before, during, and after exposure to KYN. Responses were obtained at the points marked by corresponding numbers and arrows in the graph (*bottom*). *B*: graph of PSP amplitude as a function of time during the experiment. After a brief control period, KYN was bath applied during the period indicated by the lower bar (7.5 min). Amplitude of the evoked PSP was decreased within 3 min in KYN and was completely suppressed within 5–6 min. Recovery was detectable at 4–6 min into the wash period and nearly complete when the final PSP was obtained (trace 5; in control medium for 25 min). Membrane potential was held near −75 mV under current-clamp to prevent evoked discharge.

Constant-voltage electrical stimuli were applied extracellularly through bipolar electrodes consisting of 90% platinum-10% iridium, Teflon-coated wires (76-μm diam). The stimulating electrode was placed in the region dorsolateral to the SON (for details of placement, see Fig. 1*A* and text of Dudek and Gribkoff 1987). The stimulation and recording sites were chosen to optimize the probability of activating a hypothetical cholinergic input to SON (Hatton et al. 1983). In most experiments, several stimulus intensities were employed to produce responses ranging from just suprathreshold to supramaximal. Three to five stimuli were presented at each intensity. Interstimulus intervals averaged ~2 s; synaptic plasticity, expressed as a use-dependent facilitation or depression of responses, was not observed for these stimuli. In experiments with stimulus trains, frequencies of 5–20 Hz for 3–10 s were used.

Analyses of the effects of antagonists on spike afterdischarges produced by single dorsolateral stimuli consisted of counts of the number of action potentials during the response under each experimental condition. If the membrane potential was variable at resting values, the cell was current clamped at or near the initial level. Spike threshold and input resistance were monitored throughout each experiment. Responses to stimulus trains included a depolarization [resulting from excitatory postsynaptic potential (EPSP) summation] and spike discharge during the period of stimulation; changes in these response components were noted for each antagonist.

In most experiments, the effects of antagonists were assessed on evoked PSPs. If stimulation produced action potentials at or near resting potential, the cell was current clamped at −75 to −80 mV to reveal the underlying postsynaptic potential (PSP). In some cases, an input-output plot was produced by applying multiple stimuli at five to eight voltage values, and this relationship was examined in control and drug media. For direct comparison between cells, responses at a single voltage level, corresponding to 50–80% of the voltage producing the maximal evoked amplitude, were collected under each experimental condition; the PSP amplitudes were then measured and compared. The effects of drugs on spontaneous PSPs were examined in most cells; in a select group of cells in which the frequency of spontaneous PSP occurrence was high and fairly constant, and in which these events were well resolved, the effects of KYN or DGG were quantified and an amplitude-frequency analysis was produced for the control and drug conditions. The effects of drug solutions on resting membrane potential and input resistance were examined in all cells.

Drugs used in this study were KYN (0.25–3.0 mM), DGG (0.5–2.0 mM), DL-AP5 (0.1–1.0 mM), bicuculline methiodide (BIC, 0.05–0.1 mM), *d*-tubocurarine (dTC, 0.1–1.0 mM), GABA (1.0–3.0 mM), NMDA (0.1 mM), hexamethonium bromide (HEX, 0.5 mM); all were obtained from Sigma Chemical (St. Louis, MO). Dihydro-β-erythroidine (DBE, 0.1–1.0 mM) was a gift from Merck, Sharpe & Dohme Research Laboratories.

After a control period of stable recording, at least one of the transmitter antagonists was applied to each cell, and many cells had several drug solutions applied during prolonged recordings. When possible, postdrug periods of superfusion (wash) with control solution were used to assess recovery.

RESULTS

Data for this study were obtained from 35 cells. The mean resting membrane potential in control medium was

−64.4 ± 1.1 (SE) mV (range −56 to −80 mV); the mean input resistance was 219 ± 11 (SE) MΩ (range 138–344 MΩ). At resting potential, the response to electrical stimulation dorsolateral to the SON was predominately excitatory (except 3 cells had a hyperpolarizing response); several action potentials superimposed on an EPSP often followed the stimulus. When current clamped at a membrane potential more negative than or equal to −75 mV, cells responded with a depolarizing PSP that rarely resulted in action potentials. These evoked PSPs had rise times < 5 ms and were of variable duration to ~100 ms. During stimulus trains, a maintained depolarization with superimposed small-amplitude PSPs, which resembled spontaneous synaptic events, invariably occurred; after the train, the depolarization and enhanced PSP frequency persisted for several seconds to several minutes, as described previously (Dudek and Gribkoff 1987). The effects of drug solutions were assessed on these measures of synaptic excitation and on spontaneous PSP amplitude and frequency.

Effects of KYN on synaptic potentials and afterdischarges

In all of the 19 neurons to which it was applied, KYN (0.25–3.0 mM) produced a reversible reduction of all excitatory response components. In every cell tested (n = 18), the evoked PSP was reduced in KYN. The available data suggest a concentration-dependence of this antagonism (Table 1). Complete antagonism of evoked PSPs was observed in three experiments and only at concentrations of ≥1.0 mM. A residual component of the PSP almost always persisted at high stimulus intensities. When PSPs were greatly reduced, KYN produced little or no consistent change in either membrane potential or input resistance; in some cells, a small hyperpolarization (<6 mV) was observed with KYN ≥ 1.0 mM. When the cells were returned

TABLE 1. *Effects of KYN on evoked PSP amplitude and input resistance*

	Concentration, mM				
	0.25	0.50	1.0	2.0	3.0
PSP amplitude	77 (1)	66 ± 4 (4)	45 ± 9 (11)	35 ± 15 (4)	0 (1)
Input resistance	115	91 ± 9	102 ± 5	100 ± 5	87

Values are percent of control ± SE. Numbers in parentheses are the number of cells contributing to the average response. KYN, kynurenic acid; PSP, postsynaptic potential.

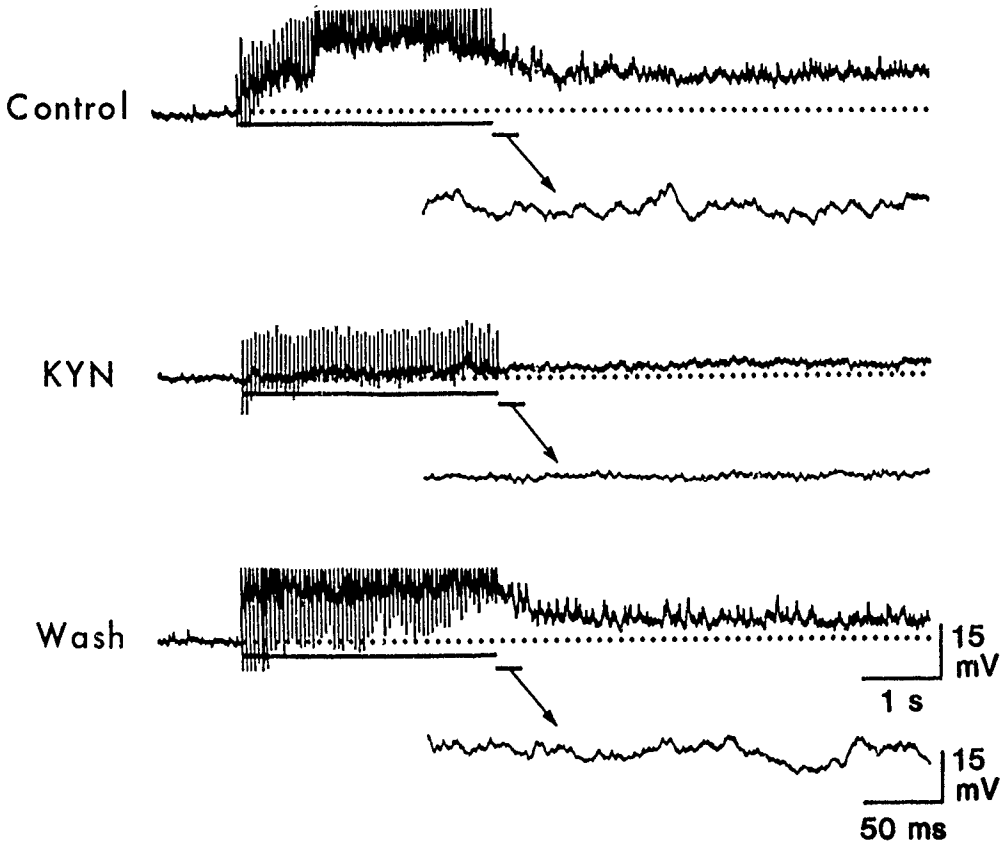

FIG. 2. Antagonism of the response to repetitive stimulation by KYN. Stimulation electrode was located in the perinuclear region dorsolateral to the SON. In control medium, a brief stimulus train (20 Hz, 3.3 s, 50 V) produced a large envelope of membrane depolarization during the period of stimulation, followed by persistent depolarization and a burst of PSP-like events. To resolve these fast events, an area (lower short solid lines) was expanded (lower traces indicated by arrows); summation prevented clear resolution of individual PSPs in this cell. In KYN (1.0 mM), the depolarization during and after the stimulus train was virtually eliminated, and the PSP burst and slow depolarization after the cessation of stimulation was likewise nearly eliminated. After return to control medium, all response components recovered to near pre-KYN levels.

to control medium for 10–60 min (n = 14), PSP amplitude partially or completely recovered. An example of KYN depression of single evoked PSPs is shown in Fig. 1, which also indicates the time course of action of KYN (2.0 mM). The time course of KYN action was examined in three cells, and a similar relationship was observed. Recovery was slower than PSP blockade.

If the resting membrane potential of a cell was less negative than or equal to −60 mV, or if a cell with a more negative resting potential was current clamped with depolarizing current to approximately −60 mV, single stimuli produced afterdischarges of action potentials, which could last 0.5 s. In every cell tested, KYN (0.5–2.0 mM) produced a rapid (n = 7) and reversible (n = 4) decrease or elimination of the afterdischarge. When the underlying evoked PSP was also monitored by current clamping the cell below spike threshold (−75 to −80 mV, n = 6), the decrease in the afterdischarge corresponded to a KYN-induced depression of the underlying PSP. The envelope of depolarization and resulting discharge produced by stimulus trains was also greatly reduced by KYN (Fig. 2). These depolarizations were probably the result of PSP summation during the 20-Hz stimulus train and were a measure of synaptic efficacy in this system. KYN (0.5–2.0 mM) concurrently reduced or abolished the in-train depolarizing response and single evoked PSPs in all eight cells tested.

Another prominent feature of the effects of KYN on the response of SON cells was seen immediately after repetitive dorsolateral stimulation (Fig. 2). In control medium, a pronounced and enduring burst of events followed the cessation of repetitive stimulation. On the basis of waveform and amplitude, they appeared to be spontaneous PSPs (see Dudek and Gribkoff 1987 for further discussion). Application of medium containing KYN produced a pronounced reduction or blockade of these events in all cells tested (n = 8). The effect of KYN on this burst of events appeared at least as great as the effect on in-train depolarization, although quantification was difficult. Because it was difficult to separate antagonism of fast PSPs after repetitive stimulation from the concurrent slow depolarization, no systematic attempt was made to determine the effects of antagonists on posttrain slow depolarizations. However, these depolarizations were reduced in several instances by KYN.

All cells recorded in this study had spontaneous depolarizing PSPs, and KYN produced a depression of spontaneous synaptic events. The effect of KYN on spontaneous depolarizing PSPs was examined closely in six cells where their amplitude (i.e., resolution) and frequency were sufficient for quantification. Figure 3 shows the typical result of KYN application (1.0 mM) on spontaneous PSPs; all events clearly identifiable as having the waveform of a PSP were grouped according to amplitude and their frequency recorded for each group over a 5-s interval before, during, and after KYN. Synaptic events in all of the amplitude groups were significantly and reversibly reduced or eliminated in KYN. The largest depolarizing events were more affected than were the smaller ones; because of the limits of resolution of this analysis technique, it was not possible to determine if the reduced number of spontaneous PSPs represents a decrease in presynaptic firing, a reduction in amplitude of PSPs such that many of these became too small to distinguish from noise, or a combination of both. The total elimination of PSPs of the largest amplitude in KYN (Fig. 3A) suggests that the amplitude distribution was shifted to the left (i.e., KYN produced a reduction in amplitude).

These six cells were analyzed in a similar manner to determine the average amplitude and frequency of all spontaneous PSPs during the analysis periods (5 s) in control medium and in the presence of KYN. For four neurons, to which 1.0 mM KYN was applied for 10–25 min, the average amplitude of all spontaneous PSPs in the

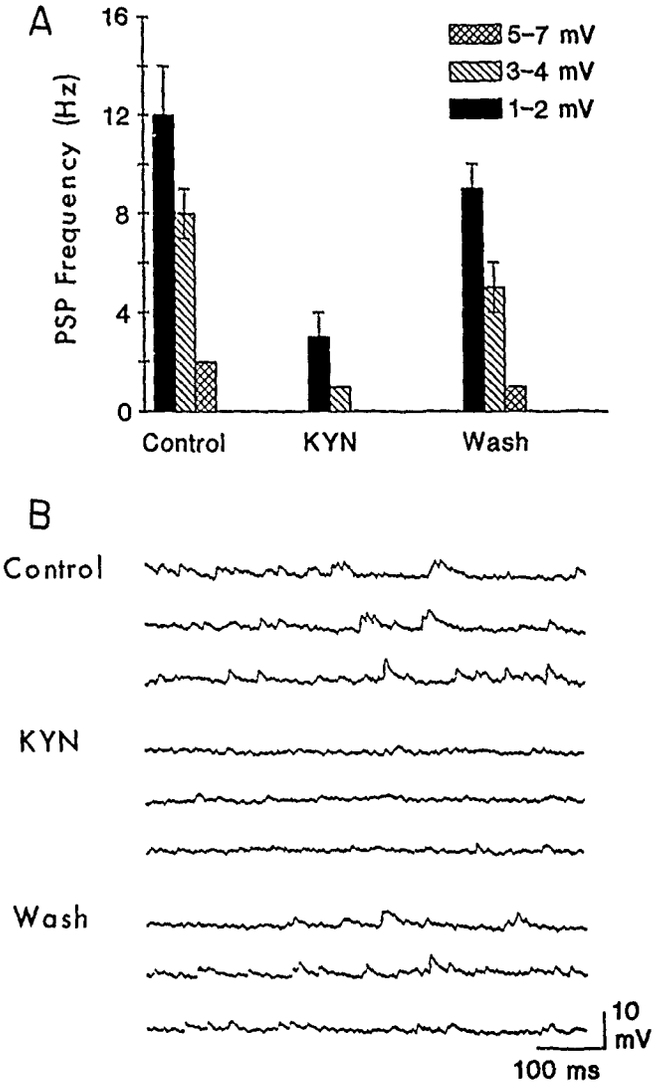

FIG. 3. Effects of KYN on spontaneous EPSPs. A: amplitude-frequency histogram for a single SON cell, displaying the effect of KYN (1.0 mM; 10 min) on the mean frequency (±SE) of spontaneous EPSPs in 3 amplitude ranges (amplitude rounded up to the nearest millivolt) during a 5-s bin. Cell had been previously bathed in BIC (100 μM; 20 min) to eliminate the possible contribution of reversed IPSPs (cell was current clamped at a membrane potential near −80 mV). KYN (also in the presence of BIC) clearly and reversibly decreased the frequency of spontaneous EPSPs at each of the amplitude ranges. Control solution partially restored the pre-KYN amplitude-frequency profile. B: examples of spontaneous EPSPs obtained from the cell contributing to the data in A. The 3 traces under each condition are continuous. Note that large-amplitude events were entirely eliminated in the presence of KYN and that they were again present after the removal of the EAA antagonist.

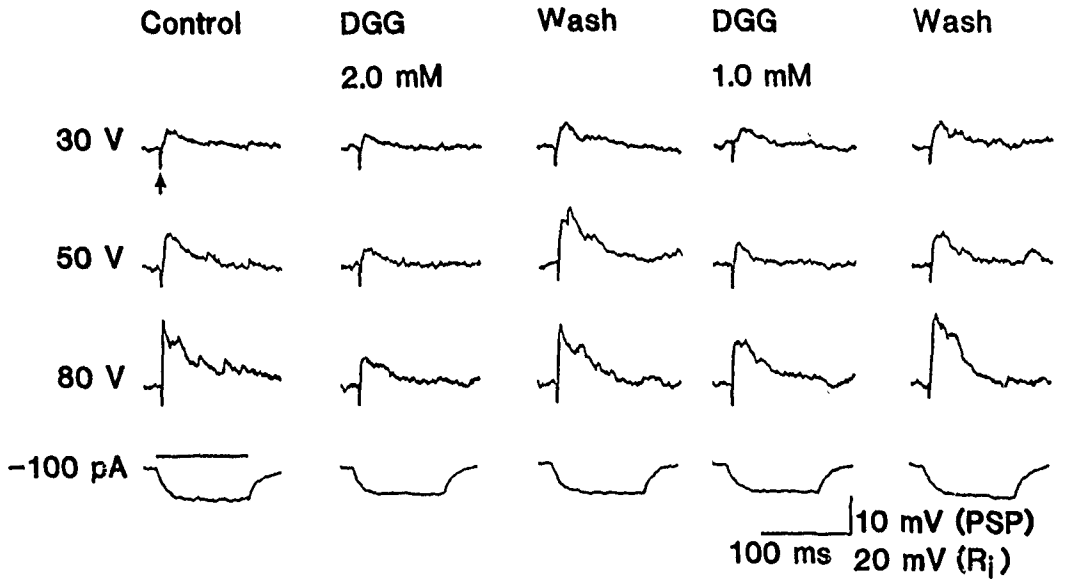

FIG. 4. Effect of DGG on evoked PSPs. Application of DGG reduced the amplitude of evoked PSPs; 2.0 mM (applied first) had a greater effect than 1.0 mM. In this cell, current clamped at a membrane potential near −75 mV, DGG at 2.0 mM moderately antagonized evoked PSPs and had no effect on input resistance (R_i). KYN (2.0 mM) completely and reversibly suppressed these potentials (not shown).

presence of the antagonist was 52 ± 7% (SE) of control levels. This compares closely with the reduction of evoked PSPs to 45 ± 9% of control under the same conditions (Table 1). The average frequency of spontaneous depolarizing PSPs was reduced to 15 ± 4% of control for these four cells. For two additional cells to which 0.5 mM KYN was applied, their amplitude was reduced to 79 ± 6% of control values. Again, this value compares reasonably well with the reduction of evoked PSPs to 66 ± 4% of control in this KYN concentration (Table 1). The average reduction in frequency of these events was less than that seen for KYN at 1.0 mM, but the SE with only two values was very high (reduced to 62 ± 41% of control). Although these data are limited, they suggest a concentration-dependent reduction of spontaneous PSP amplitude and, perhaps, frequency.

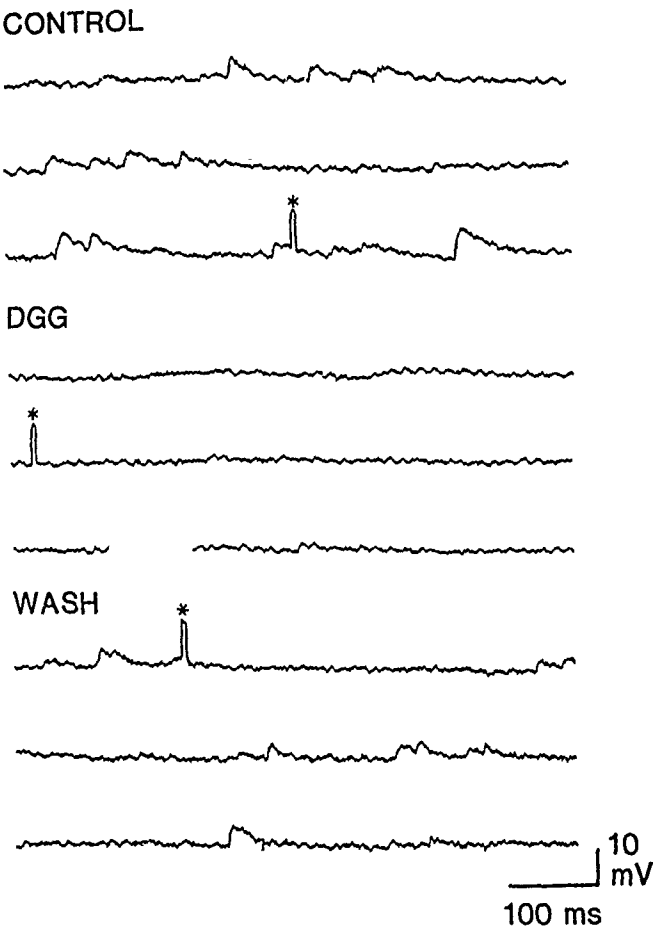

FIG. 5. Effect of DGG (1.0 mM) on spontaneous PSPs. The 3 traces under each experimental condition are continuous; positive deflections marked by asterisks are 10-mV, 5-ms calibration pulses. In control medium, PSPs of several amplitudes were observed at moderate frequency; in DGG (20 min) the PSPs were much less frequent. DGG reduced the average amplitude of remaining spontaneous PSPs to 65% of control. In this neuron, KYN (1.0 mM, 13 min) reduced spontaneous PSP amplitude to 36% of control. After a return to control medium (Wash; 15 min), the PSPs were again present at a frequency approaching that observed in control medium. Both dTC (100 μM) and BIC (100 μM) were present throughout the experiment.

Effects of DGG on evoked synaptic responses and spontaneous PSPs

To further determine whether the actions of KYN on the synaptic activation of SON neurons were due to a specific antagonism of EAA-mediated neurotransmission, another broad-spectrum EAA antagonist, DGG, was applied to six SON neurons under similar experimental conditions. The application of DGG (1.0 mM) resulted in a rapid and reversible reduction of synaptically evoked spike afterdischarges (n = 3); equimolar KYN entirely eliminated the afterdischarges or reduced them to a single action potential. The effect of DGG on evoked PSPs was examined in five cells (1.0 mM, n = 5; 2.0 mM, n = 1). DGG (1.0 mM

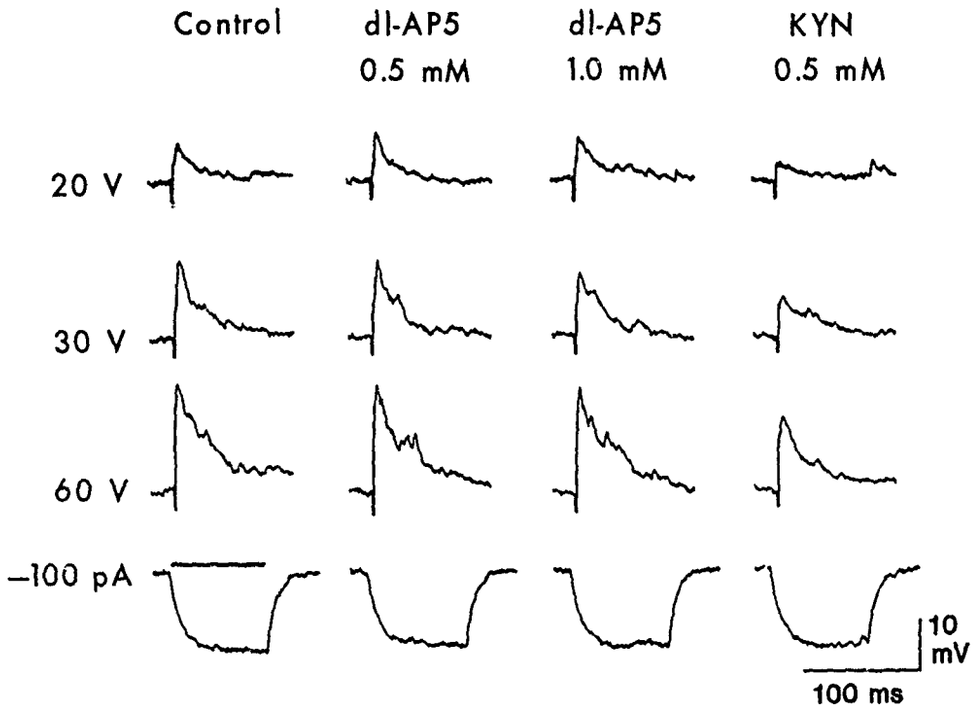

FIG. 6. Comparison of the effects of the specific NMDA-receptor antagonist AP5 and the broad-spectrum antagonist KYN on evoked PSPs. Even at high concentrations, AP5 (0.5 and 1.0 mM) did not appear to reduce PSP amplitude; KYN (0.5 mM) depressed evoked PSPs at all stimulus voltages. Neither AP5 nor KYN had an effect on input resistance, as indicated by the voltage response to a −100-pA hyperpolarizing current pulse. Note that small-amplitude, fast events (observed particularly after near-maximal stimuli) were also unaffected by AP5 and were antagonized by KYN.

for 10–25 min) produced a reversible reduction of the evoked PSP observed, as previously, at stimulus intensities that produced responses between 50 and 80% of the maximal amplitude. Figure 4 shows examples of the effect, in a single neuron, of 1.0 and 2.0 mM DGG on evoked PSPs at near-threshold to supramaximal stimulus intensities. As with KYN, this antagonist reduced PSPs at all levels of the input-output relationship. The antagonist reduced PSP amplitudes in the five experiments with 1.0 mM DGG to 64 ± 7% of the control values. DGG did not consistently

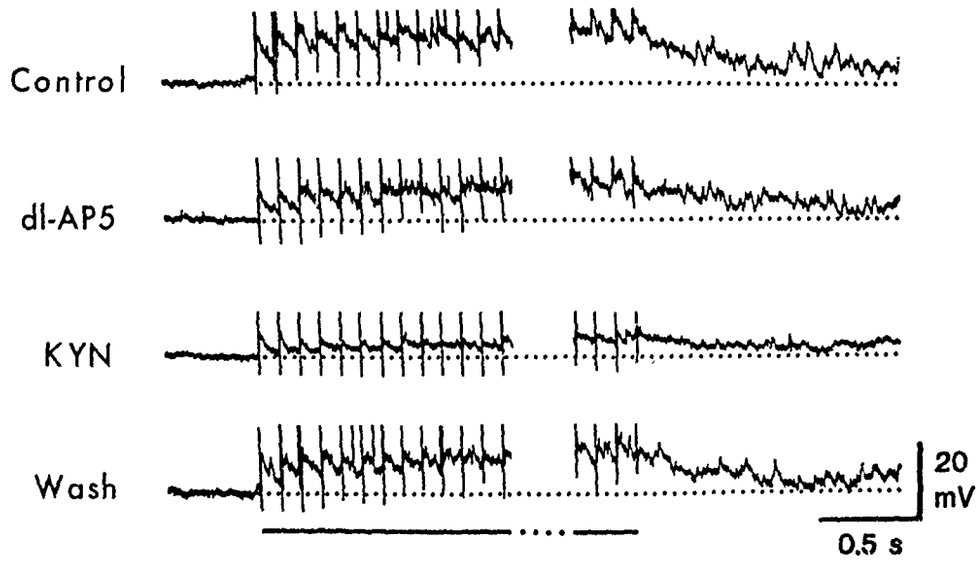

FIG. 7. Effects of AP5 (200 μM, 15 min) and KYN (1.0 mM, 15 min) on the responses to repetitive stimulation. Brief stimulus trains (10 Hz, 10 s, 60 V; indicated by line beneath *bottom trace*, entire train not shown) delivered to the dorsolateral region evoked PSPs, a summated envelope of depolarization during the train, and a burst of fast events after the train. KYN (but not AP5) greatly attenuated these response components. Residual slow depolarization was reduced but not eliminated by KYN. Return to control medium (Wash, 15 min) restored the response.

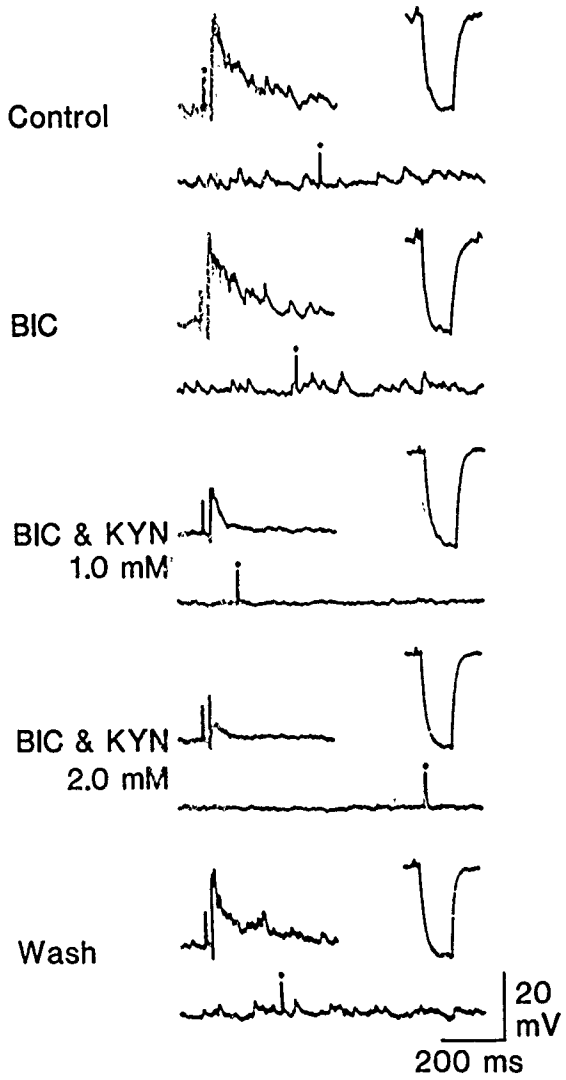

FIG. 8. Effects of BIC and KYN on PSPs and input resistance. Application of BIC (100 μM, 20 min) alone did not reduce evoked (*top left* traces) or spontaneous (*bottom traces*) PSPs, but BIC (100 μM) combined with KYN (1.0 mM, 20 min) greatly depressed the evoked PSP and eliminated spontaneous synaptic events. A higher concentration of KYN (2.0 mM, 20 min, together with BIC, 100 μM) further antagonized the evoked PSP. Responses in *top right corner* under each condition indicate that neither BIC nor KYN produced a consistent change in input resistance, as indicated by the absence of changes in the amplitude of the voltage response to brief intracellular current pulses (−100 pA, 100 ms). Cell was current clamped at −80 mV.

affect input resistance. The application of DGG also consistently reduced the amplitude of spontaneous depolarizing synaptic events, and this was analyzed quantitatively in one neuron (Fig. 5). Therefore DGG was an effective antagonist of evoked and spontaneous synaptic events, but KYN appeared to have greater efficacy.

Effects of the specific NMDA receptor antagonist AP5

To test for involvement of NMDA receptors in the EAA-mediated response to dorsolateral stimulation, we applied the specific NMDA-receptor antagonist DL-AP5 to six neurons at concentrations between 0.2 and 1.0 mM

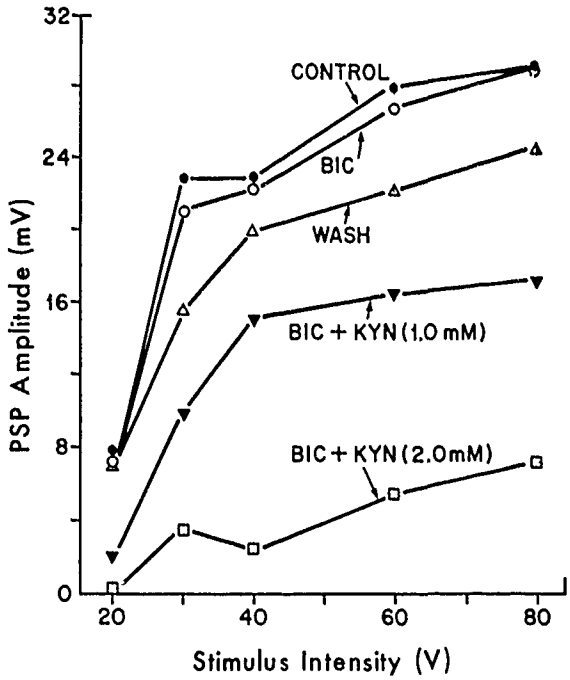

FIG. 9. Effects of BIC and KYN on responses to dorsolateral stimulation at several stimulus intensities. Values plotted are averages of 3–5 responses. This input-output relationship, generated in the cell shown in Fig. 8, demonstrates that BIC was ineffective in this neuron across the entire range of stimulus intensities used, and shows that KYN (simultaneously applied with BIC) produced a proportionately greater block at lower intensities. A substantial evoked response remained at the higher stimulus intensities, even in 2.0 mM KYN.

(because this was a racemic mixture, the effective concentration was approximately one-half that actually delivered). This antagonist produced no consistent effects on either membrane potential or input resistance in this range of concentrations.

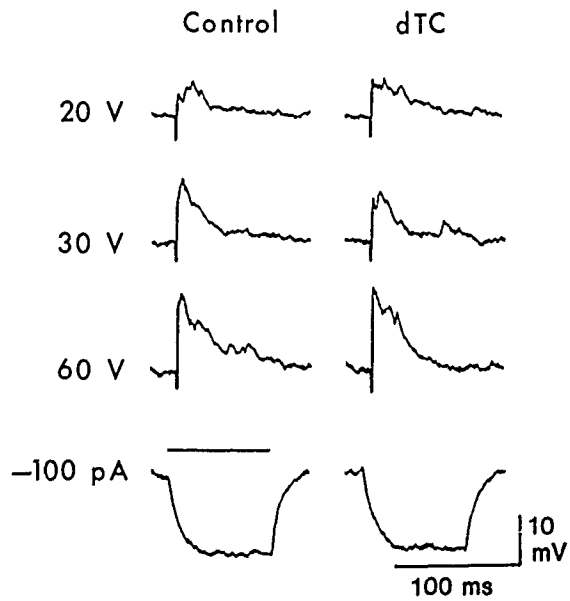

FIG. 10. Application of the nicotinic cholinergic antagonist dTC (200 μM, 25 min) did not reduce PSPs elicited by dorsolateral stimulation. Stimuli were applied at several voltages, and dTC was ineffective at every stimulus level. Neuron was current clamped to about −80 mV.

When AP5 was applied, even at high concentrations, no consistent antagonism of synaptic potentials or afterdischarges was observed (Fig. 6). In two cells, NMDA (100 μM) was applied before and during AP5 perfusion. In both cases, AP5 (0.25 and 1.0 mM) completely antagonized the excitatory actions of NMDA, although evoked and spontaneous PSPs were unaffected by the antagonist. In four cells to which AP5 was applied at a concentration of 0.2 mM, the evoked depolarizing PSP was 108 ± 3% of control values. At higher concentrations, only small reductions in amplitude were observed (83% of control, 0.25 mM, n = 1; 91% of control, 0.5 mM, n = 1; 88%, 1.0 mM, n = 2 both values equal). In three cells, AP5 (0.2–1.0 mM) also had no consistent effect on the depolarization during high-frequency dorsolateral stimulus trains (1–10 s, 3–20 Hz). In one case, shown in Fig. 8, AP5 slightly reduced the depolarization during the train, but this effect was small compared with KYN. This was also true for the fast depolarizing PSPs after the train (Fig. 7), and no effect was observed on comparable spontaneous events (n = 2).

Effects of BIC

The inhibitory transmitter GABA was probably not responsible for excitatory components of the response to dorsolateral stimulation at resting membrane potential when recordings were made with K^+-acetate–filled microelectrodes. Most cells, however, were current clamped at a membrane potential greater than or equal to −75 mV, values below the reversal potential for GABA-mediated inhibitory postsynaptic potentials (IPSPs) (see Randle et al. 1986). Therefore the degree to which reversed IPSPs contributed to evoked PSPs was tested directly. In four experiments in which 100 μM BIC was applied to the preparation, evoked PSPs remained practically unchanged (84.8 ± 13.9% of control). In 50 μM BIC, the values for two cells

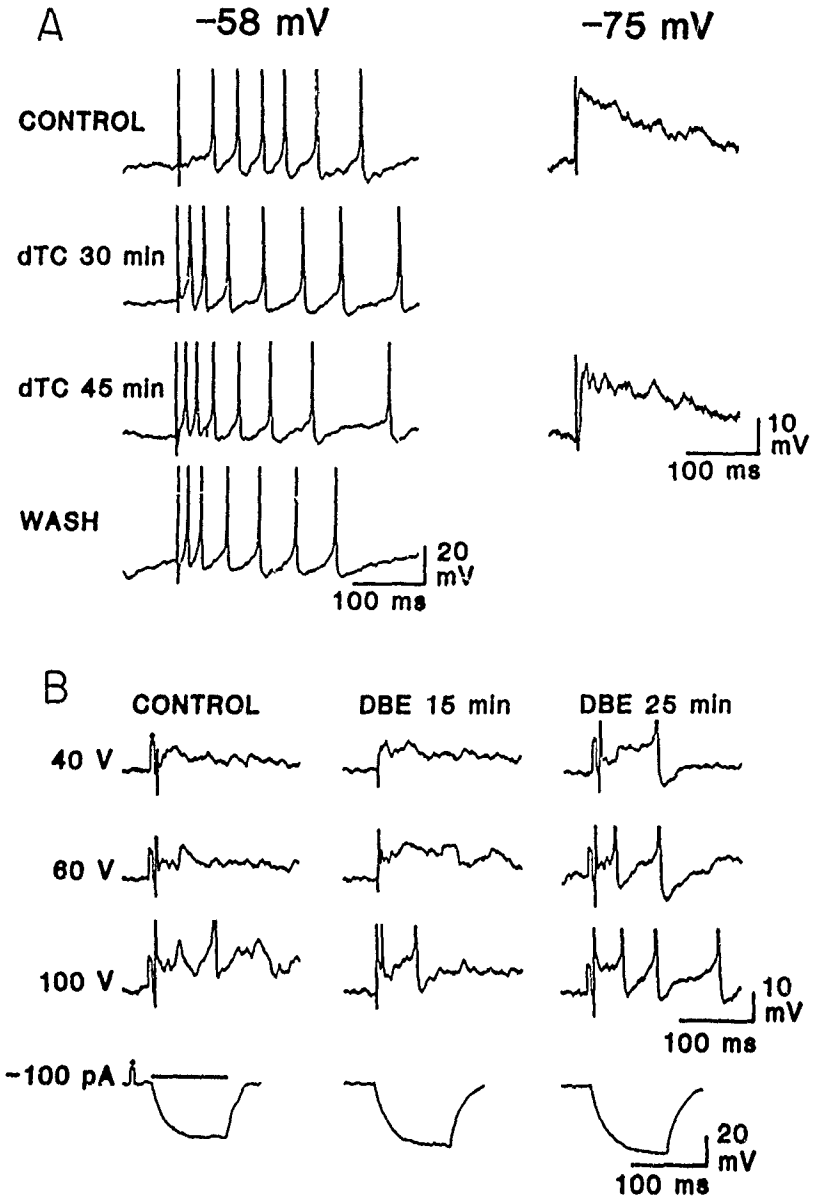

FIG. 11. Nicotinic cholinergic antagonists did not reduce evoked neuronal afterdischarges. *A*: prolonged application (>30 min) of dTC (1.0 mM) produced no detectable decrement of stimulus-evoked afterdischarge at resting membrane potential (−58 mV) or on evoked PSPs when the cell was current clamped at a more negative membrane potential (−75 mV). *B*: similar application of DBE (1.0 mM) produced no depression of responses evoked at several stimulus intensities. First downward deflection in each trace was the stimulus artifact. This cell was current clamped at a membrane potential of −72 mV throughout the experiment. Action potentials are truncated.

were 76% and 101% of control PSP amplitudes. Therefore, at these concentrations, BIC either had no effect or only slightly reduced the amplitude of the evoked PSP. In three cases in which KYN (1.0 mM) was applied in the presence of 50 or 100 μM BIC, KYN reduced the PSP significantly more than BIC alone ($X_{BIC} = 91 \pm 7\%$; $X_{BIC+KYN} = 40 \pm 11\%$; $P < 0.05$, $t = 3.94$) (see Figs. 8 and 9). Synaptically evoked afterdischarges were observed in two cells before and during the addition of BIC; in both cases no reduction in the afterdischarge was noted, and the responses appeared to have increased in intensity in BIC.

The inhibitory neurotransmitter candidate GABA was applied to two neurons to determine whether 100 μM BIC was sufficient to antagonize the effects of GABA on SON cells. In both cells, BIC antagonized the conductance increase produced by GABA. We also determined whether KYN blocked the response to GABA. Although BIC (100 μM) greatly reduced the action of GABA on input resistance, KYN (in the absence of BIC) had no effect on the GABA-induced conductance increase.

Other evidence also suggests that KYN had no effect on GABAergic transmission in the SON. In at least five neurons, addition of KYN revealed IPSPs after stimulation, where only depolarizing PSPs had been previously observed. The EPSPs antagonized by KYN appeared to have been masking the concurrently evoked small-amplitude repetitive IPSPs, and these became apparent in KYN.

Effects of nicotinic cholinergic antagonists

An earlier report indicated that stimulation of the region dorsolateral to the SON should result in activation of a population of cholinergic neurons projecting into the SON (Hatton et al. 1983). The actions of acetylcholine, secreted by this pathway, on SON cells appeared to involve an interaction with nicotinic cholinergic receptors. To test for the presence of a significant nicotinic cholinergic component in the response to dorsolateral stimulation, we applied the neuromuscular blocking agent dTC (100 μM–2.0 mM) to 10 neurons. In addition, dBE was applied to two neurons, and the ganglionic blocker HEX was applied to one cell.

A significant effect of these drugs was never observed on PSP amplitude, stimulus-evoked afterdischarge, or depolarizations and discharges during a stimulus train. The lack of effect of dTC on evoked PSPs was found across a range of stimulus intensities and dTC concentrations (e.g., 200 μM, Fig. 10). Even after prolonged exposure to high concentrations of dTC, no reduction of any response component was observed (e.g., 1.0 mM, Fig. 11*A*). Likewise, dBE (1.0 mM) did not reduce synaptic excitation (Fig. 11*B*). For the five neurons to which dTC was applied at concentrations between 200 μM and 1.0 mM and that met other inclusion criteria (see above for KYN), evoked PSPs after 10–30 min were 102 ± 8% of control amplitudes (±SE). In one experiment, HEX (250 μM) was applied to a cell for 30 min with no effect on synaptic responses. In every case in which a burst of small amplitude PSPs was observed after a stimulus train, the application of nicotinic cholinergic antagonists had no effect on this response component. Therefore nicotinic cholinergic antagonists were ineffective in antagonizing these responses.

DISCUSSION

Comparison between the effects of EAA antagonists in the SON and other systems

The EAA antagonists KYN and DGG have been shown to be specific blockers of EAA-induced excitations and synaptic potentials at several central loci. These areas include neocortex (Tsumoto et al. 1986), various hippocampal subdivisions (Collingridge et al. 1983a,b; Cotman et al. 1986; Crunelli et al. 1983; Ganong et al. 1983), spinal cord (Davies and Watkins 1981; Elmslie and Yoshikami 1985), and olfactory bulb (Jacobson et al. 1986). The tryptophan metabolite and quinoline derivative KYN and the dipeptide DGG are both broad-spectrum EAA antagonists (Francis et al. 1980; Jones et al. 1984; Watkins et al. 1987). In hippocampus, where these compounds have been intensely studied, they were shown to suppress EAA excitations mediated by agonists acting at NMDA, quisqualate, and kainate receptor subtypes, particularly at higher concentrations (Collingridge et al. 1983a,b; Crunelli et al. 1983; Ganong and Cotman 1986). In the SON, we have found KYN and DGG to be effective antagonists of evoked PSPs, afterdischarges, and spontaneous PSPs; KYN appeared to be more effective than DGG when tested on the same neuron.

As in hippocampus and spinal cord (Collingridge et al. 1983a; Elmslie and Yoshikami 1985), the antagonists began to block neurotransmission in the SON within 2–3 min and were maximally effective within ~5–8 min; the effects were reversible, although reversal was slower than the initial suppression. Comparisons of effective concentrations between SON and other areas are difficult, because in many previous studies the antagonists were applied by microiontophoresis and were often tested against the excitations produced by exogenous EAA application (e.g. Bioulac et al. 1978; Collingridge et al. 1983a). Nevertheless, in studies where these compounds were applied in the bath, millimolar concentrations produced significant antagonism (Elmslie and Yoshikami 1985; Ganong et al. 1983, 1986) and were similar to concentrations we found to be effective in the SON. The effectiveness of KYN may have been underestimated because of the presence of reversed IPSPs in the SON (see below).

An important reason for our choice of KYN and DGG over other broad-spectrum EAA antagonists was evidence from previous studies demonstrating that these compounds did not significantly interact with other central neurotransmitters and did not have nonspecific actions on cellular excitability. In particular, the antagonists were ineffective blockers of cholinergic PSPs (Elmslie and Yoshikami 1985) or excitations produced by acetylcholine (Collingridge et al. 1983b). A previous study had suggested that the PSPs produced in SON neurons by dorsolateral stimulation were cholinergic (Hatton et al. 1983), making the issue of cholinergic interactions by EAA antagonists an important selection criterion, because antagonist effects on

cholinergic excitations were not directly tested in this study. In addition, these compounds have not previously produced significant nonspecific effects on cell membrane properties. This was also true in SON; KYN and DGG did not alter significantly input resistance. The small-amplitude hyperpolarizations in KYN probably resulted from the profound blockade of spontaneous depolarizing synaptic events. This has been directly demonstrated in the spinal cord (Elmslie and Yoshikami 1985). DGG did not appear to produce changes in membrane potential.

While the antagonism of evoked responses by KYN and DGG implicated EAAs in the mediation of these events, their ability to interact with all EAA receptor subtypes did not allow us to determine the identity of the particular receptor(s) responsible. In particular, both compounds appeared, at least in hippocampus, to antagonize excitations mediated by NMDA-receptor activation (Collingridge et al. 1983b; Ganong and Cotman 1986). Because specific antagonists of quisqualate- and kainate-type EAA receptors were not available at the time the experiments were conducted, we utilized the specific NMDA antagonist AP5 to determine whether this receptor subclass was involved in the fast excitatory responses to dorsolateral stimulation. Little or no effect of AP5 was observed for PSPs in the SON, even at high concentrations, which strongly suggests that NMDA-receptor activation was not a mechanism of PSP generation in these neurons under these experimental conditions. It is worth noting, however, that the excitatory response of SON neurons to bath-applied NMDA, and its blockade by high concentrations of AP5, suggests the presence of NMDA receptors in SON.

Effects of KYN and DGG on spontaneous PSPs and responses to stimulus trains

Spontaneous PSPs were seen in all cells recorded in SON, although their frequency and amplitude varied considerably between cells. KYN and DGG greatly reduced the average amplitude and frequency of these events. Whereas the frequency of all such events was reduced, larger amplitude spontaneous PSPs were absent in the presence of these compounds. A similar shift in the amplitude distribution of spontaneous PSPs in KYN has been observed in hippocampal neurons (Cotman et al. 1986). This action of KYN was suggested to represent a primary postsynaptic action on EAA receptors, rather than an effect on presynaptic discharge, which would affect the frequency but not amplitude distribution. Our data, which show a disproportionate reduction of larger amplitude events, can be interpreted in a similar manner. We suggest that KYN and DGG reduced the amplitude of spontaneous PSPs via an interaction with non-NMDA-type EAA receptors on magnocellular neurons, because AP5 did not affect spontaneous PSPs. Apparent reductions in overall spontaneous PSP frequency may have reflected a reduction of the more frequent low-amplitude events, which became impossible to resolve from base-line noise.

Events similar in amplitude and frequency to spontaneous PSPs occurred at high frequency after stimulus trains delivered to the region dorsolateral to the SON and were greatly reduced by KYN. These events possibly resulted from stimulation of local circuit neurons, which secrete EAAs. Alternatively, these events may have resulted from stimulation of afferents that originated outside the slice, and were the reflection of the repetitive firing of axons or the release of large transmitter "quanta." These arguments apply equally well to spontaneous PSPs. Our experiments could not differentiate between these hypotheses.

The sustained depolarizations observed during repetitive stimulation were reduced or eliminated by KYN and DGG. These depolarizations probably resulted from PSP summation during the train, and their reduction by KYN and DGG resulted from antagonism of the fast PSPs. The residual slow depolarizations after the trains (Dudek and Gribkoff 1987) were also reduced by KYN and DGG, although this effect was not quantified. The origin of these long-lasting depolarizations is unknown, but their reduction by broad-spectrum EAA antagonists suggests that the depolarization was produced directly or indirectly by EAAs, possibly involving an additional mechanism, such as an increase in the extracellular concentration of K^+ resulting from cellular discharge.

GABAergic component of evoked PSPs

In a previous study, dorsolateral stimulation produced a mixture of excitation and inhibition (Hatton et al. 1983), and, during this study, data became available that demonstrated GABA involvement in inhibitory neurotransmission in the SON (Randle et al. 1986). Although in our hands dorsolateral stimulation almost always produced excitation, the application of KYN at depolarized membrane potentials revealed previously undetected IPSPs in some cells. We therefore applied the GABA antagonist BIC to determine whether there was an unlikely interaction between KYN and GABAergic neurotransmission and to assess the percentage of evoked PSPs (recorded at hyperpolarized membrane potentials) that represented a depolarizing GABAergic component. Such depolarizing responses could have resulted from current clamping the cells below the reversal potential for GABA (Randle et al. 1986). The ~15% reduction of PSPs in 100 μM BIC suggested a small GABAergic component in the response under these experimental conditions; the presence of IPSPs in KYN and the inability of KYN to reduce GABA-induced conductance increases strongly argues that KYN did not antagonize a GABAergic component. These data suggest that the efficacy of KYN at antagonizing EAAs in SON may have been underestimated. Preliminary data from comparable experiments in the paraventricular nucleus support this idea (Wuarin and Dudek 1988).

Cholinergic neurotransmission in the SON

A previous study (Hatton et al. 1983) has strongly implicated acetylcholine, through an interaction with nicotinic receptors, in the response of SON neurons to electrical stimulation dorsolateral to the SON. Cholinergic neurons are located in the dorsolateral region (Mason et al. 1983; Meeker et al. 1988; Theodosis and Mason 1988), and Hat-

ton and colleagues (1983) found that nicotinic antagonists, generally applied by drop into a "microwell," depressed the extracellularly recorded afterdischarge that resulted from dorsolateral stimulation. Although we positioned our stimulating electrodes in the same approximate area and used a slice similar to that described and diagrammed by these investigators, we did not find any effect of nicotinic cholinergic antagonists on intracellularly recorded responses. However, we have found that nicotine can excite SON cells, and this effect can be blocked by dTC (Gribkoff et al. 1988). Although it remains a possibility that other nicotinic cholinergic antagonists may prove more effective in blocking the PSPs, at least two lines of evidence suggest that this is unlikely. First, recent studies argue that the cholinergic cells previously thought to innervate the SON may not project into this nucleus and that cholinergic neurotransmission in the region dorsolateral to the SON may be mediated via a muscarinic receptor (Meeker et al. 1988; Theodosis and Mason 1988). Second, our data with EAA antagonists would leave little room for a significant role for cholinergic involvement in SON neuronal responses evoked by dorsolateral stimulation. The broad-spectrum EAA antagonists blocked most of the evoked responses; if nicotinic cholinergic interaction does occur, it would be limited to a minor KYN-resistant, BIC-resistant component. The results of the previous study (Hatton et al. 1983) may have been complicated by possible nonspecific effects of cholinergic antagonists, comparable with the antagonism of GABAergic responses by dTC (Lebeda et al. 1982). This could have been further complicated by the unknown final concentration of antagonists when applied by the microdrop method.

In conclusion, the data obtained in these experiments point to an EAA as the primary excitatory neurotransmitter in the SON. As opposed to previous indications that acetylcholine was responsible for excitatory events evoked by local stimulation (Hatton et al. 1983), both evoked and spontaneous excitatory synaptic responses were blocked only by specific EAA antagonists. Although the same stimuli may also have activated the dorsolateral cholinergic neurons, we found no evidence for a significant cholinergic projection to SON from this area.

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Biomedical Research 10, Supplement 2, 00-00, 1989

EXCITATORY AMINO ACIDS MEDIATE FAST SYNAPTIC TRANSMISSION IN THE HYPOTHALAMUS

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ABSTRACT

This review deals with the mechanisms responsible for fast excitatory synaptic transmission in the hypothalamus. We present data supporting the hypothesis that glutamate, or a closely related amino acid, is the primary transmitter responsible for fast excitatory input to hypothalamic neurons, including neuroendocrine cells. The key result to support this hypothesis is that excitatory amino acid (EAA) antagonists consistently block both evoked and spontaneous excitatory postsynaptic potentials (EPSPs) in the supraoptic, paraventricular and suprachiasmatic nuclei. Recent data obtained with selective EAA antagonists suggest that non-NMDA receptors mediate fast synaptic transmission in these hypothalamic nuclei. Further research is required to test this hypothesis for other cell types in the mammalian hypothalamus.

Excitatory Amino Acids as Transmitters in the Hypothalamus

Two fundamental questions in mammalian neuroendocrinology are ; 1) which substances mediate synaptic transmission in the hypothalamus, and 2) how similar are synaptic mechanisms in the hypothalamus to those in other areas of the mammalian brain ? Until recently, there were no experimental tests of the hypothesis that EAAs comprise a major neurotransmitter system in the hypothalamus. For example, in several reviews dealing with the neurotransmitters and neuropeptides that regulate secretion of neurohypophyseal hormones (24, 27, 28, 30), little or no discussion was given to EAAs. Most hypothalamic neurophysiologists have apparently assumed that acetylcholine, acting on nicotinic cholinergic receptors, is the most important transmitter responsible for fast excitatory synaptic transmission. Although acetylcholine, norepinephrine, and a variety of peptides are probably important endogenous regulators of hypothalamic hormone secretion, we propose that their contribution to fast synaptic transmission is limited or non-existent. The hypothesis to be developed in this review is that *glutamate, or a similar EAA, is the primary transmitter mediating fast excitatory synaptic transmission in the mammalian hypothalamus.*

One experimental strategy in identifying hypothalamic neurotransmitters has been to test the responses of postsynaptic neurons to locally applied neuroactive substances and to match them to the postsynaptic response following stimulation of the neural pathway. However, more convincing evidence can be obtained when antagonists for the putative neurotransmitter are used to block synaptic events (*e.g.* post-

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synaptic potentials) evoked by stimulation of the neural pathway. High-quality intracellular recordings of synaptic events and application of antagonists to synaptic sites are extremely difficult *in vivo*, but slice or explant preparations allow these experiments by virtue of their inherent stability and accessibility. In this chapter, we will consider the effects of specific EAA antagonists on synaptic potentials. We believe this is an *essential* approach for transmitter research. Recent studies on synaptic potentials have been directed at the roles of excitatory and inhibitory amino acids, such as glutamate (9, 10) and gamma-aminobutyric acid (GABA) (26).

Supraoptic and Paraventricular Nuclei

Probably the most intensely studied neuroendocrine system of the mammalian hypothalamus, at least in terms of electrophysiologic properties, is the magnocellular neuroendocrine system (7, 27, 28). The supraoptic and paraventricular nuclei, which comprise this system, are responsible for secretion of vasopressin and oxytocin. The supraoptic nucleus primarily contains oxytocinergic and vasopressinergic neuroendocrine cells that project to the neurohypophysis. The paraventricular nucleus has a more diverse population of neuropeptidergic cells, which project not only to the neurohypophysis but also to the median eminence and other central nervous system structures. Slices and explant preparations have contributed to major advances in our understanding of the membrane properties and the mechanism of action of transmitters and neuromodulators on hypothalamic neuroendocrine cells. In both supraoptic and paraventricular neurons, spontaneous and evoked excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) can be recorded intracellularly. These physiological events presumably arise from either 1) axons whose distant cell bodies were disconnected during preparation of the tissue, and/or 2) local neurons, which have intact short-axon projections to these neurons. The primary strategy outlined here has been to analyze the effects of EAA antagonists on intracellularly recorded EPSPs.

Suprachiasmatic Nucleus

Another region of the hypothalamus that has received considerable attention in terms of the mechanisms of synaptic transmission is the suprachiasmatic nucleus. The main reason that so much research has been performed in this area is that a large body of evidence suggests that the suprachiasmatic nucleus plays a key role in the regulation of circadian rhythms. Neurobiological studies have focused on the retinal input to the suprachiasmatic nucleus, which is the anatomical substrate for photic entrainment and phase shifting of the circadian rhythm. Several reports using extracellular recording with broad-spectrum EAA antagonists (*e.g.* kynurenic acid and gamma-D-glutamylglycine) have provided evidence suggesting that glutamate is the transmitter for the retinal projection to the suprachiasmatic nucleus. Although this research has been an important first step, extracellular recordings have severe interpretational limitations. The two published reports of intracellular recordings provided preliminary data on the electrophysiological properties of suprachiasmatic neurons (31, 34), but they did not deal with mechanisms of synaptic transmission. We have initiated an intracellular analysis of the retinal input to the suprachiasmatic nucleus with a focus on evaluating the effect of EAA antagonists on EPSPs from optic nerve stimulation (18).

METHODS

We will only provide a brief outline of the techniques used in this research. The reader is referred to the primary literature cited at the end of this article (*e.g.* 7). In our laboratory, the supraoptic and paraventricular nuclei have been studied with coronal slices of approximately 500 μm thickness. The work on the suprachiasmatic nucleus has also included horizontal and parasagittal slices, which have allowed selective stimulation of the optic nerve. We have used relatively standard techniques for intracellular recording from hypothalamic neurons. Our approach in these studies has involved bath application of EAA antago-

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nists and an examination of their effects on EPSPs.

RESULTS

Supraoptic Nucleus

Electrical stimulation of a site dorsolateral to the supraoptic nucleus evokes EPSPs and afterdischarges in most supraoptic neurons (6, 13). Several lines of indirect evidence suggested that acetylcholine, acting on nicotinic cholinergic receptors, mediates transmission from neurons in the dorsolateral region to vasopressinergic neurosecretory cells in the supraoptic nucleus (13). We undertook research aimed at testing this hypothesis by studying the effects of nicotinic cholinergic antagonists on intracellularly recorded EPSPs (10). In preliminary studies, however, we found that acetylcholine, nicotine and carbamylcholine increased the firing rate of about half of the supraoptic neurons; this could be blocked with the nicotinic antagonists, D-tubocurarine and hexamethonium (8), thus confirming that nicotinic cholinergic receptors are present on some supraoptic neurons. However, bath-applied D-tubocurarine did not affect the EPSPs evoked by electrical stimulation dorsolateral to the supraoptic nucleus (10). No clear decrement in synaptic responses was detectable, even with very high doses of D-tubocurarine and even though we were previously able to show that this antagonist blocked the direct responses to acetylcholine and nicotine. Deadwyler and colleagues were also unable to find any effects of bath-applied hexamethonium on the EPSPs of supraoptic neurons in response to dorsolateral stimulation, even at high concentrations (personal communication). These data are inconsistent with the hypothesis that cholinergic neurons in the area dorsolateral to the supraoptic nucleus are responsible for the EPSPs in magnocellular neuroendocrine cells. The lack of effect of nicotinic cholinergic antagonists on the EPSPs of supraoptic neurons strongly suggested that another class of transmitter mediates the fast EPSPs in this system.

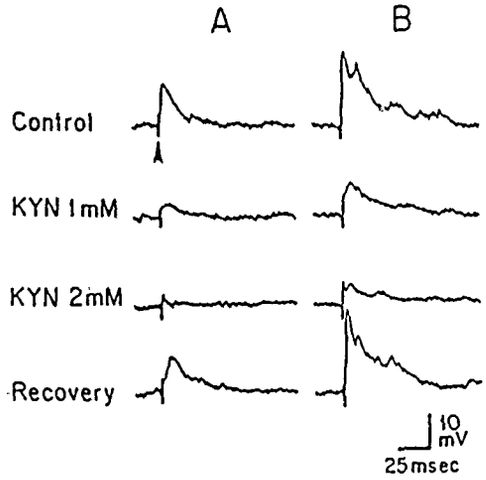

Fig. 1 Effect of kynurenic acid on evoked EPSPs of a supraoptic neuron. The control EPSPs to single stimuli (arrow) at moderate (A; 20V) and near-maximal intensity (B; 30V) in the area dorsolateral to the supraoptic nucleus are shown before bath-application of kynurenic acid (KYN). The antagonist was applied at 1 mM and 2 mM for 15 min. The EPSPs to both intensities of stimulation were depressed, and the effect of 2 mM kynurenic acid was greater than 1 mM. When kynurenic acid was washed out of the recording chamber, the EPSPs recovered to their original amplitude. Steady hyperpolarizing current was injected into this neuron in order to current clamp the membrane potential at −80 mV. Reproduced from ~~(9)~~, with permission of Elsevier Science Publishers.

In the last several years it has become increasingly clear that EAAs are an important neurotransmitter system throughout the entire mammalian central nervous system (19, 32). The development of specific EAA antagonists has been a crucial component of this research. For example, when kynurenic acid or gamma-D-glutamylglycine (1-2 mM) were bath-applied while recording intracellularly from supraoptic neurons, both afterdischarges and EPSPs (Fig. 1) evoked from stimulation dorsolateral to the nucleus were strongly attenuated (9, 10). Similar effects were also seen on spontaneous EPSPs. Although it has been difficult to bath-apply several concentrations of these antagonists to each cell and obtain reversible effects for each concentration, analyses across cells strongly suggested that the effects were

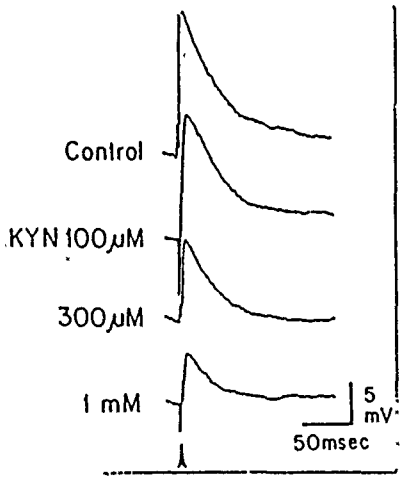

Fig. 2 Depression of EPSPs of a paraventricular neuron by kynurenic acid. Extracellular electrical stimuli near the fornix (arrow) caused EPSPs in a paraventricular neuron. The EPSPs were evoked during a hyperpolarizing current pulse, which is not shown. Each trace is the average of 20 responses. The preparation was bathed in 50 μM picrotoxin, which eliminated IPSPs. Kynurenic acid reduced the EPSP amplitude in a concentration-dependent manner. Data from (55), and reproduced from (7), with permission from Elsevier Science Publishers.

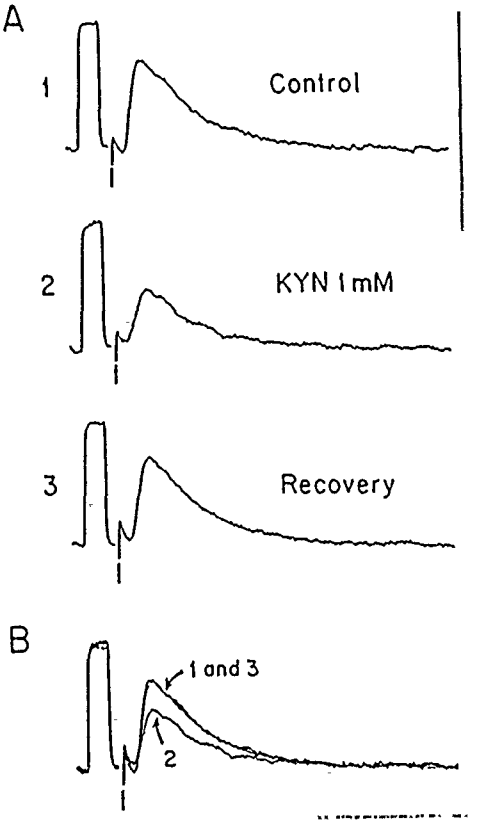

Fig. 3 Effect of kynurenic acid on EPSPs of a suprachiasmatic neuron. The EPSPs were evoked by electrical stimulation of the contralateral optic nerve. Each of the three traces is an average of 10 responses. Steady hyperpolarizing current was injected into this neuron to current clamp membrane potential below threshold. Kynurenic acid (1 mM) reduced the EPSP amplitude by >30%. A calibration pulse (10 mV, 10 ms) preceded the optic nerve stimulus (arrow). Both individual (A) and superimposed traces (B) are shown. Data from (16).

dose-dependent. These antagonists did not affect resting membrane potential, spike threshold or input resistance. The selective *N*-methyl-D-aspartate (NMDA) antagonist, DL-2-amino-5-phosphonopentanoic acid (AP5), had little or no effect on synaptic responses, at least under these recording conditions. These data provide strong evidence that EAAs represent a widespread and important transmitter system in the supraoptic nucleus. Recent preliminary studies by Dribkoff and VandenMaelen (11) using the recently developed quinoxalinediones, which are potent competitive non-NMDA receptor antagonists (17), have confirmed the importance of EAA receptors in the generation of these synaptic events and have demonstrated that non-NMDA receptors mediate these potentials.

Paraventricular Nucleus

The added complexity of the paraventricular nucleus, both in terms of the cell types and their projections, required that these issues be addressed separately in this nucleus. Several recent studies have begun to define the electrophysiologic properties of paraventricular neurons and to relate these properties to the anatomy and immunohistochemistry of this region (15, 16, 23, 33). After studying the electrophysiologic properties of the neurons, we bath-applied kynurenic acid to neurons in and around the paraventricular nucleus (35). Experiments were performed in pi-

crotoxin to eliminate contamination of EPSPs from simultaneous activation of IPSPs mediated by $GABA_A$ receptors and chloride channels. The EPSPs were signal-averaged to optimize signal-to-noise ratio and to thereby obtain more accurate dose-response data. We found that kynurenic acid consistently blocked EPSPs from stimulation of the perifornical area (Fig. 2). Recent experiments with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) strongly support the hypothesis that non-NMDA receptors mediate these EPSPs (36). Taken together, these data indicate that EAAs are an important fast excitatory transmitter system in the paraventricular nuclei, as well as the supraoptic nucleus.

Suprachiasmatic Nucleus

Although several studies had suggested that EAAs mediate transmission from the retina to the suprachiasmatic nucleus (1-3, 29), we have undertaken experiments with intracellular recording aimed at addressing this issue in more detail. We found that kynurenic acid depressed EPSPs to optic nerve stimulation (Fig. 3) with little or no effect on passive or active membrane properties. In addition, 6,7-dinitroquinoxaline-2,3-dione (DNQX) potently and reversibly depressed EPSPs to optic nerve stimulation and to stimulation of local sites around the suprachiasmatic nucleus (18). These studies were also performed in bicuculline and have used a variety of slice orientations to ensure specific electrical stimulation of the optic nerve with no contamination from other afferents. Ongoing studies are aimed at defining which types of neurons in the suprachiasmatic nucleus, both in terms of their electrophysiology and anatomy, receive retinal and non-retinal synaptic input mediated by EAAs.

DISCUSSION

Excitatory Amino Acid Antagonists

We have reviewed recent data from several investigations demonstrating that EAA antagonists strongly and consistently depress EPSPs in the supraoptic, paraventricular and suprachiasmatic nuclei. This effect of EAA antagonists on EPSPs has been seen with virtually every cell that has been recorded from each of these nuclei. Although one could argue that the broad-spectrum antagonists (*i.e.* kynurenic acid and gamma-D-glutamylglycine) require millimolar doses, a large body of previous research suggests that these antagonists do not affect other transmitter receptors. Furthermore, the effects of the quinoxalinediones have been obtained with concentrations in the micromolar range. Although more work is needed, these collective data strongly suggest that EAAs may mediate *all* fast EPSPs throughout the hypothalamus. Additional recordings in other hypothalamic nuclei, combined with electrical stimulation of other synaptic inputs, are required to test this hypothesis more rigorously.

Potential Criticisms

Several possible criticisms can be proposed against this hypothesis, but none of them seems very well-supported. For example, audioradiographic binding studies have reported relatively few EAA binding sites in certain hypothalamic areas compared to telencephalic structures (5, 20, 21, 25). However, this technique is a *relative* measure of binding, and the electrophysiological data suggest that although relatively few EAA receptors are present in the hypothalamus compared to other brain areas like hippocampus and neocortex, they are still important for synaptic transmission. Similarly, several neurotoxicity studies with various EAAs have shown relative sparing of certain hypothalamic neurons and/or areas (12, 14, 22). However, the resistant neurons may be less vulnerable to excitotoxic damage because of lower numbers of EAA receptors (particularly NMDA receptors) and/or differences in second-messenger systems (4). These data do not argue against an important role for EAAs in synaptic transmission in the hypothalamus.

Prospects for Future Research

The data reviewed here suggest several new avenues of future research. First, the potency and selectivity of the quinoxalinediones as non-NMDA receptor antagonists provide the possibility of evaluating in intact animals the role of EAAs in specific neuroendocrine processes, such as lactation (*i.e.* the milk ejection reflex) and parturition. The synaptic component of vasopressin secretion from osmotic stimuli could also involve input from afferent fibers that use an EAA as a transmitter. Other studies could be aimed at determining whether EAA antagonists block the effects of photic stimulation on circadian rhythms.

A second line of future research relates to the physiological roles of other transmitters and neuromodulators. Robust activation of synaptic inputs that use EAAs may confound the analysis of other transmitter systems, and in particular, may obscure or contaminate slow synaptic potentials. When EAA antagonists are used to block fast EPSPs, it may be possible to detect slow synaptic events for other important modulatory inputs to the hypothalamus.

CONCLUSION

This review has dealt with a series of neuropharmacologic and electrophysiologic studies aimed at identifying the primary transmitter substances responsible for fast synaptic transmission in the hypothalamus. The data considered here, along with the work of Randle, Bourque and Renaud (26) on GABAergic inputs to supraoptic neurons, strongly suggest that excitatory and inhibitory amino acids are the transmitter systems responsible for fast EPSPs and IPSPs in all hypothalamic neurons. Although further research is needed to test this hypothesis more rigorously, we believe that the results from recent experiments with EAA antagonists require a major revision of our views on the role of EAAs (*e.g.* glutamate) in hypothalamic transmission.

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THE EFFECTS OF OSMOLALITY ON SYNCHRONOUS BURSTING IN THE ABSENCE OF CHEMICAL SYNAPTIC TRANSMISSION IN HIPPOCAMPAL SLICES.

J.G. Tasker and F.E. Dudek, Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Numerous studies using low-$[Ca^{2+}]$ solutions have indicated that non-synaptic mechanisms can synchronize electrical activity in the hippocampus. We examined the effects of altered extracellular osmolality on CA1 population responses after blocking chemical synaptic transmission in slices of rat hippocampus. Synaptic responses to single and repetitive electrical stimuli were completely blocked in solutions in which Ca^{2+} was replaced with EGTA (1-2 mM) and kynurenate (3 mM). Bursts of population spikes and/or negative shifts were induced in CA1 when $[K^+]$ was raised to 5 mM. When negative shifts occurred without population spikes, reduction of the extracellular osmolality by adding water (5-20%) or lowering NaCl (10-20 mM) caused bursts of population spikes. When bursts occurred in solutions of normal or lowered osmolality, addition of mannitol or sucrose (+5-40 mOsm/kg), which are membrane impermeant, dramatically reduced the bursts. Addition of glycerol (+5-40 mOsm/kg), which is membrane permeant, had little or no effect. The effects of mannitol and sucrose could be reversed by diluting the medium (i.e., decreasing osmolality). All effects of changing osmolality were at least partially reversible. Thus cellular swelling in dilute media, and the resultant reduction of the extracellular space, enhance neuronal synchrony, even in the absence of chemical synaptic transmission. Similarly, cell shrinkage from increased extracellular osmolality reduces synchrony. These data strongly support the hypothesis that electrical field effects and/or changes in extracellular $[K^+]$ play an important role in the synchronization of hippocampal neurons.

Supported by AFOSR 87-0361.

KEY WORDS: (see instructions pg. 4)

1. CA1
2. EPILEPTIFORM BURST
3. FIELD EFFECT
4. HIPPOCAMPUS

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COMPARATIVE ELECTROPHYSIOLOGY OF MAGNOCELLULAR AND PARVOCELLULAR NEURONS OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. N.W. Hoffman*, J.G. Tasker and F.E. Dudek (SPON:R.S. Fisher). Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

The hypothalamic paraventricular nucleus (PVN) contains both magnocellular and parvocellular neuronal populations, which makes selective intracellular study of either one difficult. Virtually nothing is known about the electrophysiology of PVN parvocellular neurons. We recorded intracellularly from PVN neurons (n=32) in coronal hypothalamic slices. About 60% of recorded neurons displayed Ca^{2+}-dependent low-threshold spikes (i.e., LTS cells) capable of generating 1-2 action potentials. Most LTS cells had non-linear current-voltage (I-V) relations and a long membrane time constant (22.5 ± 2.0 ms, SEM). The remaining 40% of PVN neurons showed no low-threshold spike (non-LTS cells), linear I-V relations and a shorter time constant (15.5 ± 2.0 ms). The LTS and non-LTS neurons had similar input resistances (230 ± 18 MΩ and 200 ± 25 MΩ), resting potentials (60 ± 2.5 mV and 63 ± 3.0 mV) and action potential amplitudes (62 ± 1.0 mV and 66 ± 1.5 mV from threshold). Following electrophysiological characterization, some cells were injected with biocytin (Horikawa and Armstrong, J. Neurosci. Meth., 25:1, 1988) and neurophysin immunohistochemistry was performed (n=7). Two of 3 injected non-LTS neurons were neurophysin-positive, suggesting they were magnocellular; 4 of 4 LTS neurons were neurophysin-negative, suggesting they were parvocellular. We suggest, therefore, that magnocellular and parvocellular neurons can be distinguished based on their intracellular electrophysiology.

Supported by AFOSR 87-0361.

KEY WORDS: (see instructions pg. 4)

1. Hypothalamus
2. Paraventricular Nucleus
3. Neurophysin
4. Intracellular Stain

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CONTRASTING EFFECTS OF NMDA AND NON-NMDA ANTAGONISTS ON FAST EPSPs IN NEURONS OF THE PARAVENTRICULAR NUCLEUS. J.-P. Wuarin* and F.E. Dudek Mental Retardation Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024

Excitatory amino acids may mediate most of the fast excitatory synaptic transmission in the supraoptic nucleus (Gribkoff, V.K. and Dudek, F.E., Brain Res. 442:152, 1988). Using the paraventricular nucleus (PVN), we applied antagonists for specific amino acid receptors to determine the respective contribution of N-methyl-d-aspartate (NMDA) and non-NMDA receptor subtypes to the excitatory postsynaptic potential (EPSP) and current (EPSC). Intracellular recordings were obtained from slices of guinea-pig hypothalamus in 50 μM picrotoxin. Synaptic activation was obtained by electrical stimulation of the region dorsolateral to the fornix. The non-NMDA antagonist, 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX), induced a dose-dependent decrease of the EPSP and EPSC: 1 μM had no detectable effect, 3 μM and 10 μM produced 30% and 70% decrease respectively, and 30 μM almost completely blocked the synaptic response. The NMDA-selective antagonist, D,L-2-amino-5-phosphonopentanoic acid (AP5), applied at 30 μM did not affect EPSP or EPSC amplitude or duration even when the cell was depolarized. These results suggest: 1) non-NMDA receptors mediate fast excitatory synaptic responses within the PVN and 2) although NMDA receptors may be present on PVN neurons, they seem to be less important than non-NMDA receptors in normal synaptic transmission. These data support the hypothesis that excitatory amino acids, acting primarily or exclusively on non-NMDA receptors, are the major excitatory neurotransmitter system in the hypothalamus.

Supported by DAO5711, AFOSR87-0361, and the Swiss NSF.

KEY WORDS: (see instructions pg. 4)

1. NEUROENDOCRINE
2. NMDA
3. KAINIC ACID
4. QUISQUALATE

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ANTAGONISM OF FAST EXCITATORY POSTSYNAPTIC POTENTIALS IN SUPRACHIASMATIC NUCLEUS NEURONS BY EXCITATORY AMINO ACID ANTAGONISTS. Y.I. Kim and F.E Dudek. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

The possible role of excitatory amino acids (EAAs) in fast synaptic transmission in the suprachiasmatic nucleus (SCN) was investigated with intracellular recording. Seven SCN neurons were recorded in horizontal and coronal brain slices prepared from five male rats and two male guinea pigs, respectively. Fast excitatory postsynaptic potentials (EPSPs) were evoked by stimulating optic nerve, optic chiasm ventrolateral to the SCN or a site dorsolateral to the SCN. At resting potential, spontaneous action potentials often obscured the EPSPs. When cells were hyperpolarized 20-40 mV below threshold, depolarizing PSPs from optic nerve had mean amplitude and onset latency of 5.6 mV and 12.3 ms (n=4), while PSPs from other sites were 7.0 mV and 3.0 ms (n=6). Bath-applied kynurenic acid (1 mM), a wide-spectrum EAA antagonist, attenuated these PSPs from optic nerve (n=2) and optic chiasm (n=1) by 21-34%. In slices treated with bicuculline (a $GABA_A$ antagonist, 50 μM), 6,7-dinitroquinoxaline-2,3-dione (0.3-3.0 μM), a non-NMDA receptor antagonist, attenuated the PSPs from optic nerve (n=2), optic chiasm (n=1) and the dorsolateral site (n=2) by 15-76%. The data suggest that EAAs, presumably through non-NMDA receptors, mediate fast excitatory synaptic transmission in the SCN. This includes both retinal and non-retinal input.

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KEY WORDS: (see instructions pg. 4)

1. OPTIC NERVE
2. NMDA
3. EPSP
4. SLICE

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Osmolality-Induced Changes in Hippocampal Epileptogenesis: Importance of Non-Synaptic Mechanisms

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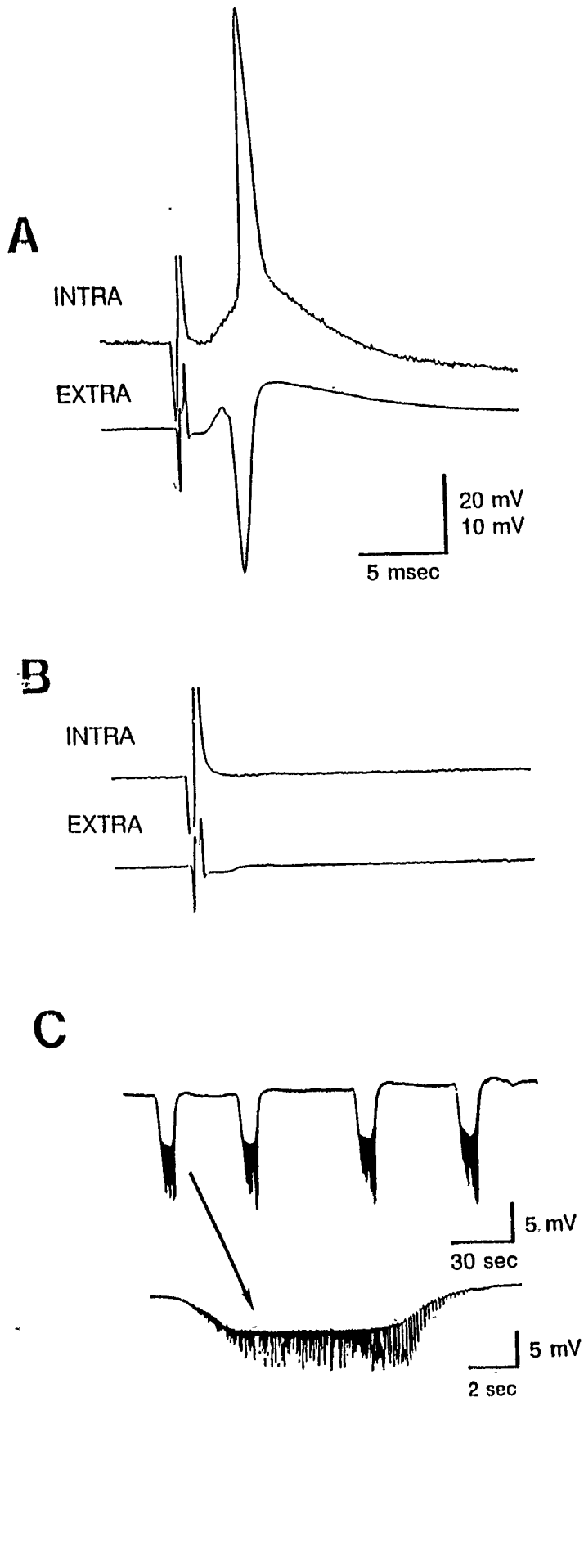
ABSTRACT

The contribution of non-synaptic mechanisms to the high seizure susceptibility of the hippocampus was examined in vitro by testing the effects of osmolality on synchronous neuronal activity, using solutions which blocked chemical synaptic transmission both pre- and postsynaptically. Decreases in osmolality, which shrink the extracellular volume, caused or enhanced epileptiform bursting. Increases in osmolality with membrane-impermeant solutes, which expand the extracellular volume, blocked or greatly reduced epileptiform discharges. Our data argue that reductions in the extracellular volume enhance the non-synaptic mechanisms of synchronization among CA1 hippocampal neurons. Since similar osmotic treatments are known to modify epileptiform discharges in several models of epilepsy, non-synaptic mechanisms are more important in hippocampal epileptogenesis than previously realized and may contribute to the high sensitivity of this brain region to hypoxic and epileptic damage in animals and humans.

Chemical synapses are clearly the dominant form of neuronal communication under normal conditions, but non-synaptic mechanisms (i.e., electrical field effects or ephaptic interactions, changes in extracellular $[K^+]$, and electrotonic coupling through gap junctions) may be critically important in abnormal states, such as epilepsy (1). Several laboratories independently showed that low-$[Ca^{2+}]$ solutions, which block chemical synaptic transmission, give rise to spontaneous bursts of synchronized activity in hippocampal slices (2); this suggests that mechanisms other than chemical synapses can synchronize the electrical activity of hippocampal neurons. However, whether chemical synapses were completely blocked and thus whether non-synaptic mechanisms are sufficient for neuronal synchronization in the hippocampus has been controversial (3). Changes in the osmolality of the extracellular fluid alter the extracellular volume (4,5) and the effectiveness of non-synaptic mechanisms of neuronal synchronization. Experiments on cortical brain slices (4,5) and on animal models of epilepsy (6) have shown that changes in the osmolality of the extracellular fluid alter epileptiform activity, but the mechanisms underlying the effects of osmolality has been unclear because chemical synapses were operative. We now test the hypothesis that the extracellular volume determines the strength of non-synaptic mechanisms of neuronal interaction and is therefore a critical factor in hippocampal epileptogenesis. This study shows directly that even when chemical synapses have been demonstrably blocked both pre- and postsynaptically, synchronized electrical activity of CA1 hippocampal pyramidal cells is still sensitive in a predictable manner to changes in the osmolality of the extracellular fluid. These data emphasize the important role that non-synaptic mechanisms play in synchronizing the electrical activity of CA1 hippocampal neurons, which may account for their high sensitivity to seizure- and hypoxia-induced damage under clinical conditions.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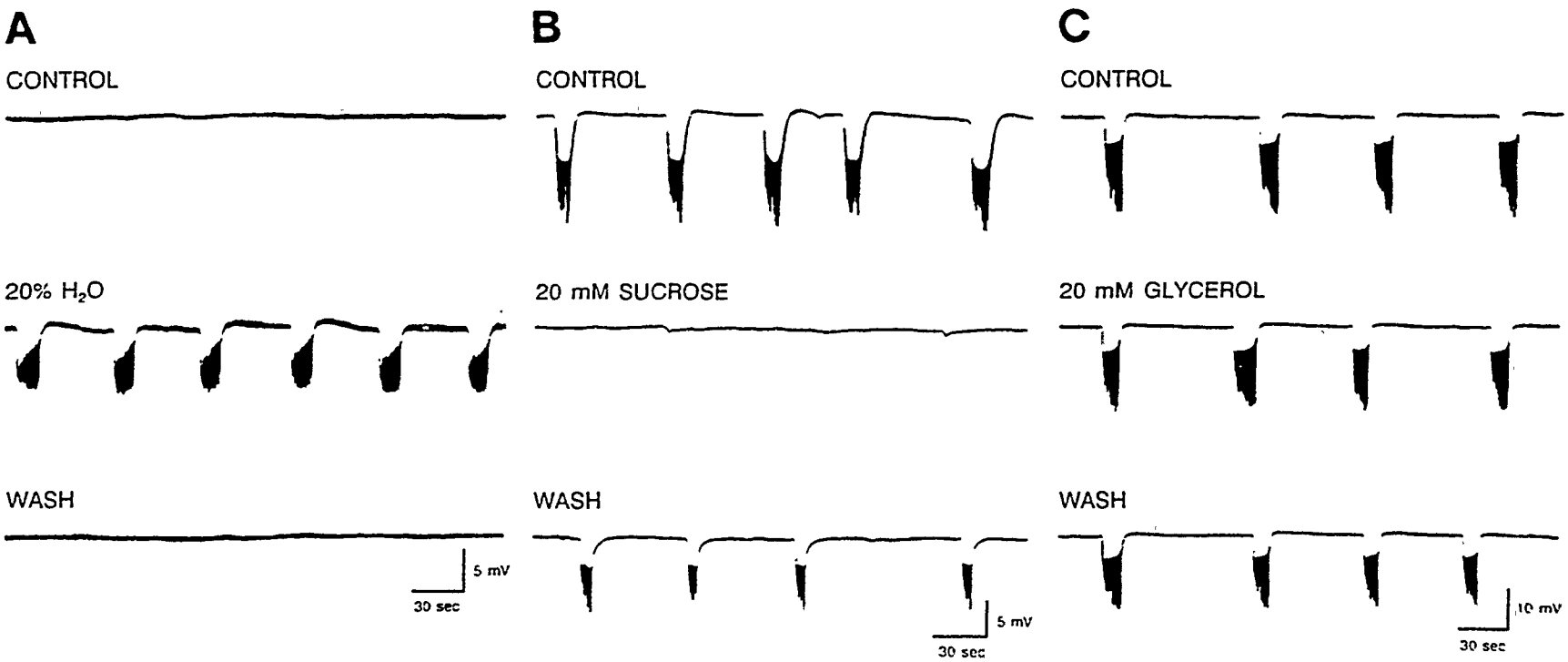
A
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20% H_2O
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WASH
5 mV
30 sec
C
CONTROL
20 mM GLYCEROL
WASH
10 mV
30 sec

The interpretation that chemical synaptic transmission is blocked in low-[Ca^{2+}] solutions has been questioned on two grounds (3). First, although low-[Ca^{2+}] solutions block synaptic responses to single stimuli (2), facilitation may occur during repetitive stimulation (7). Second, previous studies (e.g., see 2) could not rule out a possible contribution of depolarization-induced, calcium-independent transmitter release; although such a hypothetical mechanism might not cause synaptic potentials, it could lead to increased transmitter levels in the extracellular space. In the present study, we used both a low-[Ca^{2+}] solution (i.e., Ca^{2+} omitted) with 1 mM EGTA and high concentrations (30 μM) of the excitatory amino acid antagonists, 6,7-dinitroquinoxaline-2,3-dione (DNQX) and D,L-2-amino-5-phosphonopentanoate (AP5), which block the postsynaptic effects of glutamate (8). Intracellular and extracellular synaptic responses to repetitive stimulation (12 and 24 Hz) were completely blocked (Fig 1A & B). Therefore, chemical synaptic transmission was blocked presynaptically with a low-[Ca^{2+}], EGTA-containing solution and postsynaptically with excitatory amino acid antagonists.

Within 30 to 120 min after application of low-[Ca^{2+}] solution, spontaneous bursts of synchronized compound action potentials ("population spikes") usually occurred at regular intervals (Fig. 1C). Dilution of the extracellular medium (5-20% H_20), which causes cell swelling and a reduction of the extracellular volume (9), induced (Fig. 2A) or greatly enhanced epileptiform bursts in 5-10 min (N=9). Conversely, addition of a membrane-impermeant solute (i.e., 5-40 mM sucrose or mannitol), which causes cell shrinkage and a resultant increase of the extracellular volume, reversibly blocked or depressed the spontaneous epileptiform bursts in 15-30 min (N=9, Fig. 2B). Glycerol (5-40 mM), a membrane-permeant solute, had little or no effect on the bursts during a 30-min application (N=6, Fig. 2C). These experiments demonstrate that increases or decreases in the osmolality of the extracellular medium and their asssociated effects on extracellular volume, can

depress or enhance, respectively, epileptiform bursts (10). These effects occur even when chemical synaptic transmission has been unequivocally blocked. Therefore, mechanisms that depend on the size of the extracellular space and are independent of chemical synapses are responsible for the generation and/or synchronization of these epileptiform bursts.

If non-synaptic mechanisms play a major role in the synchronization of hippocampal neurons, and thus significantly augment the susceptibility of the hippocampus to epileptic seizures, then experimental alteration of the extracellular volume should affect epileptiform bursting. Previous studies have shown that changes in osmolality can alter epileptiform activity both in vitro and in vivo (4-6). Although there is evidence that the observed osmotic effects were not due to chemical synapses (4,5), it is impossible to rule out a contribution from chemical transmission in these studies. Indeed, some of the available data have suggested that these osmotic effects potentially involve or depend upon chemical synapses (5,6). In the present study, however, non-synaptic mechanisms must account for the effects of osmolality on epileptiform activity, since chemical synapses were completely blocked.

Intense activity of cortical neurons is known to cause cell swelling and concomitant shrinkage of the extracellular volume (11), similar to what would be expected in hypoosmotic solutions. Our data strongly suggest that activity-dependent reductions in extracellular volume and subsequent enhancement of non-synaptic mechanisms of neuronal excitation are part of an important positive-feedback loop that contributes to the induction and maintenance of epileptogenesis. Non-synaptic mechanisms of neuronal interaction may account for the high seizure susceptibility of CA1 pyramidal cells, and for the sensitivity of this area to seizure- and hypoxia-induced damage (12). Decreases in plasma osmolality are known to cause seizures in a variety of abnormal clinical conditions, and increases in plasma osmolality reliably block seizures in humans

(13); the basis for this effect likely depends on non-synaptic mechanisms, such as electrical field effects (i.e., ephaptic interactions) and changes in extracellular $[K^+]$.

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7. Although facilitation of synaptic responses was observed in 0.2 mM [Ca^{2+}] with no EGTA, [A. Konnerth and U. Heinemann, Neurosci. Lett. **42**, 255 (1983)], most studies on low-[Ca^{2+}] bursting (2) have used low [Ca^{2+}] plus EGTA and/or Ca^{2+}-channel blockers, such as Mn^{2+} [e.g. C.P. Taylor and F. E. Dudek, Science **218**, 351 (1982)]. Some of these studies on low-[Ca^{2+}] bursting (2) clearly showed that synaptic responses were blocked at low stimulation frequencies, but none of them consistently tested high-frequency stimulus trains. The possible unmasking of chemical

transmission late in a stimulus train could not account for the profound synchrony of action potentials that can occur early in a burst, although it could contribute to the slow changes that have primarily been attributed to shifts in extracellular [K^+] [see p. 152 & 153 of C.P. Taylor and F.E. Dudek, J. Neurophysiol. 52, 143 (1984)].

8. Numerous studies have clearly shown that these concentrations (or lower ones) of quinoxalinediones (CNQX and DNQX) and AP5 are sufficient to abolish excitatory chemical synaptic transmission and/or epileptiform bursts in the hippocampus [T. Honore et al., Science 241, 701 (1988); J.F. Blake, M.W. Brown and G.L. Collingridge, Neurosci. Lett. 89, 182 (1988); R.S. Neuman, Y. Ben-Ari, M. Gho and E. Cherubini, Neurosci. Lett. 92, 64 (1988); R.S. Neuman, Y. Ben-Ari and E. Cherubini, Brain Res. 474, 201 (1988); O. Herreras et al., Neurosci. Lett. 99, 119 (1989)]. We have also confirmed that in normal solutions, DNQX and AP5 (30 μm) abolished synaptic responses in CA1 pyramidal cells [A. Obenaus, J.G. Tasker and F.E. Dudek in preparation]. Some initial experiments (N=5) were performed in kynurenic acid (3 mM), where chemical synaptic transmission was also demonstrably blocked.

9. It is well-known that water moves into cells and causes swelling in hypoosmotic solutions, and that water moves out of cells and leads to shrinkage in hyperosmotic solutions if the added solutes are membrane impermeant [E.K. Hoffman and L.O. Simmonsen, Physiol. Rev. 69, 315, (1989)]. Membrane permeant solutes (e.g., glycerol) have no osmotic effects in the time frame of these experiments. Changes in extracellular resistance, indicative of alterations in extracellular volume, have been measured previously in the CA1 area during similar changes in osmolality (4).

10. Additional experiments have shown that the enhanced epileptiform activity in hypoosmotic solutions could be depressed with hyperosmotic solutions containing mannitol or sucrose, and vice versa [A. Obenaus, J.G. Tasker and F.E. Dudek, in preparation].

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14. Conventional methods were used for the preparation and in vitro maintenance of hippocampal slices (400 μm). The normal extracellular solution contained (in mM): 124 NaCl, 3.0 KCl, 2.4 $CaCl_2$, 26 $NaHCO_3$, 2.0 $MgSO_4$, 1.24 NaH_2PO_4 and 10 glucose. The low-$[Ca^{2+}]$ solution contained (in mM): 0 $CaCl_2$, 1.0 EGTA, 0.03 DNQX and 0.03 AP5. Intracellular and extracellular recordings were obtained from the cell body layer of CA1.

15. The osmolality of these solutions was measured with a vapor-pressure osmometer.

16. This research was supported by a grant from the Air Force Office of Scientific Research (87-0361 and 90-0056) to F.E.D. and by a National Institutes of Health postdoctoral fellowship to J.G.T. We are grateful to Drs. C. Houser, C. Wasterlain, and G. Zampighi for their constructive criticisms. We thank C. Kinney for secretarial assistance.

FIGURE LEGENDS

Fig. 1. Pre- and postsynaptic blockade of chemical synapses and subsequent development of epileptiform activity in the CA1 area of rat hippocampal slices (14). **A** Synaptic responses to electrical stimulation of stratum radiatum in normal medium. Intracellular recordings (INTRA; single response) showed a typical excitatory postsynaptic potential (EPSP) with a superimposed action potential. Extracellular field potentials (EXTRA; average of five responses) displayed a positive-going population EPSP and a faster, negative-going population spike. **B** Pre- and postsynaptic block of chemical synaptic transmission. Ca^{2+}-dependent release of synaptic transmitter from presynaptic terminals was blocked with low-[Ca^{2+}] solutions (i.e., Ca^{2+} omitted) containing 1 mM EGTA. Postsynaptic excitatory amino acid receptors were blocked with the non-NMDA antagonist, 6,7-dinitroquinoxaline-2,3-dione (DNQX, 30 μM) and the NMDA antagonist, D,L-2-amino-5-phosphonopentanoate (AP5, 30 μM) (8). Synaptic responses were consistently and completely blocked to single and repetitive stimuli (12 and 24 Hz, 0.5 ms for 10 s). The intracellular and extracellular responses are averages of the last 20 responses to 24 Hz stimulation. Calibrations are the same as in A. **C** Spontaneous bursts of population spikes after blockade of chemical synaptic transmission. Within 40 min, most slices had spontaneous bursts of population spikes in the CA1 area. During each burst, a negative shift in extracellular potential was followed by repetitive population spikes which are shown at slow (above) and fast (below) time scales.

Fig. 2. Effects of osmolality on epileptiform bursts after blockade of chemical synapses. **A** Induction of epileptiform bursts by hypoosmotic medium (20% H_2O; -35 mOsm, 15). In those slices where spontaneous bursts did not occur, dilution of the fluid reversibly induced synchronous bursts of population spikes (9). **B** Block of spontaneous epileptiform bursts with hyperosmotic medium. Addition of 20 mM sucrose (+30 mOsm), an impermeant solute, to the perfusion medium blocked epileptiform bursts. **C** Lack of effect of the permeant solute, glycerol, on spontaneous epileptiform bursts.